TRENDS IN URBAN RODENT MONITORING AND MITIGATION: IMPROVING OUR UNDERSTANDING OF POPULATION AND DISEASE ECOLOGY, SURVEILLANCE AND CONTROL

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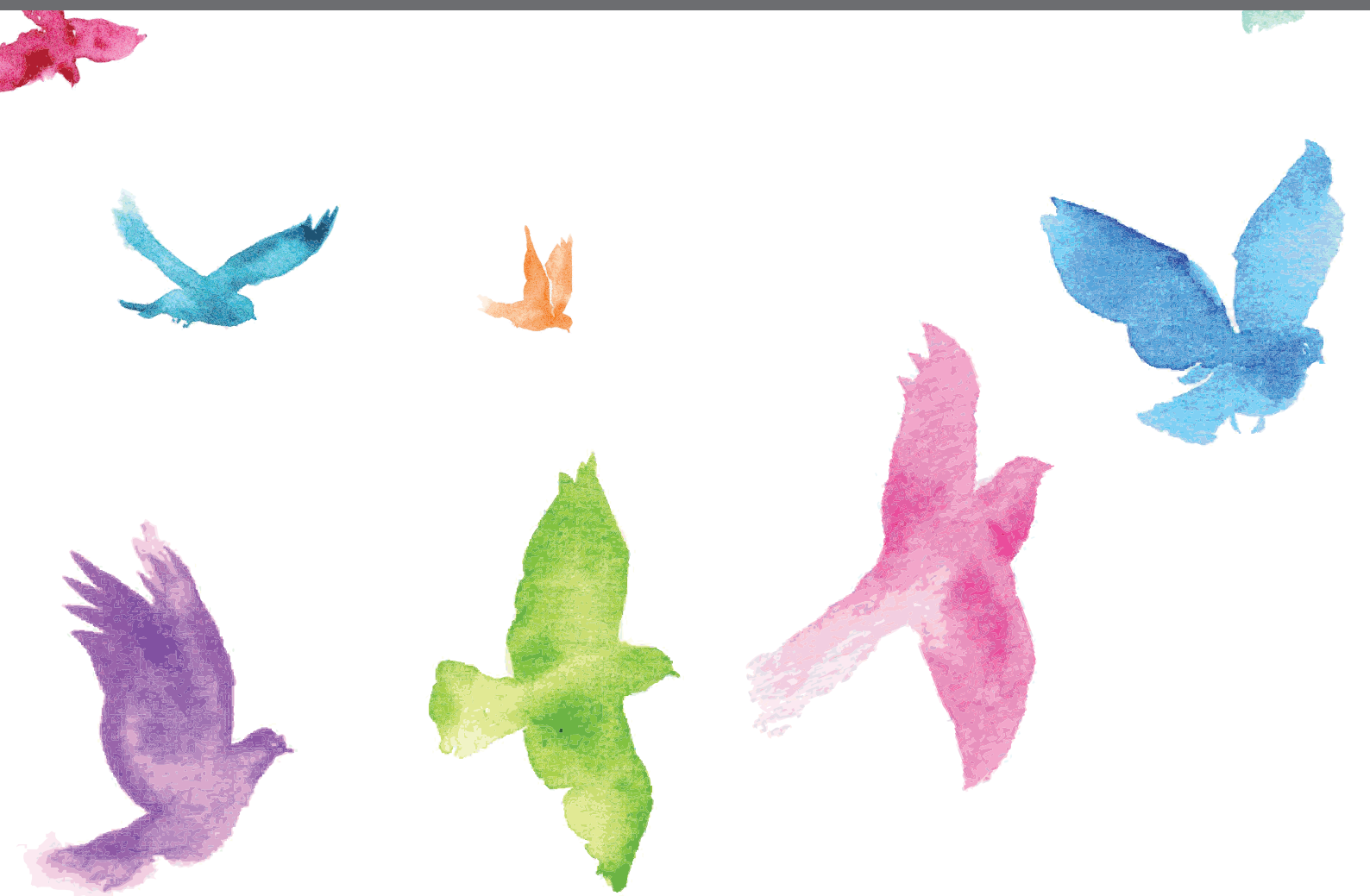

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TRENDS IN URBAN RODENT MONITORING AND MITIGATION: IMPROVING OUR UNDERSTANDING OF POPULATION AND DISEASE ECOLOGY, SURVEILLANCE AND CONTROL

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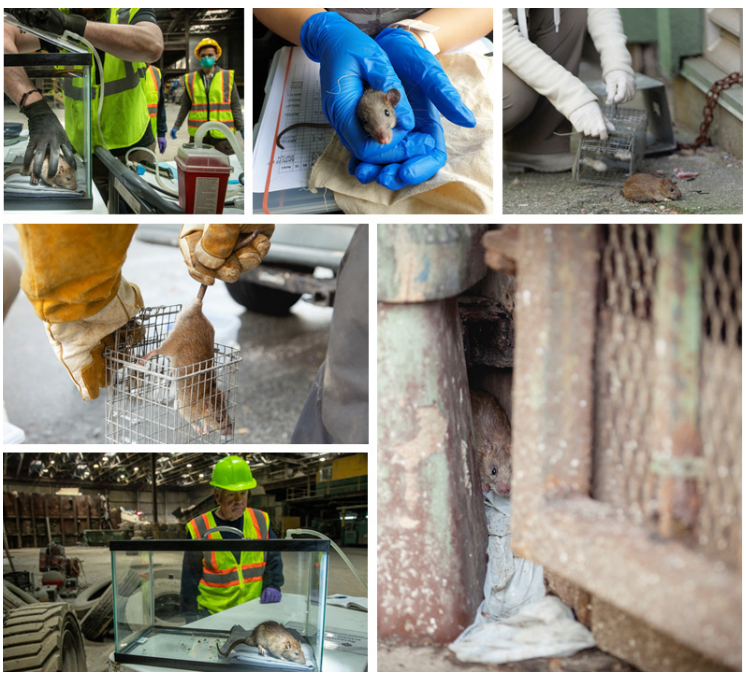

Top left: photo from Michael Parsons lab, New York City, USA. Image credit: Charlie Hamilton, National Geographic. **Top middle:** photo courtesy of Mathew Crowther lab, Sydney Australia. **Top right:** Releasing rats for a capture-mark-recapture study on the impact of lethal pest control on rat population and disease ecology. Part of the Vancouver Rat Project. Image credit: Lindsay Elliot. **Middle left:** Live capture of urban Norway rats as part of the Vancouver Rat Project. Image credit: Lindsay Elliot. **Bottom left:** photo from Michael Parsons lab, New York City, USA. Image credit: Charlie Hamilton, National Geographic. This image identifies Michael A. Deutsch, with permission, of Arrow Exterminating Inc. **Bottom right:** A Norway rat hiding in Vancouver, Canada. Part of the Vancouver Rat Project. Image credit: Lindsay Elliot.

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EDITORIAL
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Editorial: Trends in Urban Rodent Monitoring and Mitigation: Improving Our Understanding of Population and Disease Ecology, Surveillance and Control

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Keywords: city rats, pest management, rodentology, urban disease ecology, vector, wild rodents

Editorial on the Research Topic

Editorial: Trends in Urban Rodent Monitoring and Mitigation: Improving Our Understanding of Population and Disease Ecology, Surveillance and Control

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The urban environment is unique among earth's ecosystem in that it is almost entirely created, maintained, and modified by humans. As such, it is not often a focus of ecological research (Dyson et al., 2019). However, a number of wild animals thrive in urban centers, particularly rodents. Indeed, certain species of rodents are so well-adapted to close cohabitation with people that they are rarely found in habitats devoid of their human counterparts (Aplin et al., 2003). These so-called commensal rodents can be found in almost every corner of every city on earth (Lund, 1994) and humans are more likely to interact with them than any other wildlife species.

Unfortunately, these interactions can lead to a diverse array of negative consequences. For example, urban rodents carry a number of zoonotic pathogens associated with significant human morbidity and mortality (Himsworth et al., 2013). Exposure to rodents may also impact mental health, particularly among marginalized populations (Lam et al., 2018). Finally, urban rodents consume and contaminate food stuffs, damage property and infrastructure, start fires and result in significant expenditures on pest control (Feng and Himsworth, 2014). Over half of the world's population currently live in urban centers (United Nations, 2018), and given increasing rates of urbanization, these issues are likely to increase in the future. Unfortunately, there are a number of significant gaps in our understanding of urban rodents which impede our capacity to adequately prepare for current and future threats (Parsons et al., 2015).

There are several reasons for the prevailing knowledge gaps (Traweger et al., 2006; Banks et al., 2014). Many urban rodents are inherently difficult to study as they are nocturnal, secretive, and reside in habitats not readily accessible to researchers (e.g., deep within infrastructure) (Parsons et al., 2015). They disproportionately populate impoverished neighborhoods (Himsworth et al., 2013; Feng and Himsworth, 2014) where residents are disempowered to deal with rodent-related issues compared to those living in more affluent areas (Lam et al., 2018). Many societies have negative

associations with rodents (German and Latkin, 2016) and property owners may keep infestations secret because of shame, fines or possible business closures (Pimentel et al., 2005; Parsons et al., 2017).

Counter intuitively, the fact that humans have so much exposure to urban rodents may be responsible for the fact we know so little about them. Specifically, it is commonly assumed that because urban rodents are omni-present, scientists and authorities already understand all there is to know about them (Parsons et al., 2016). This attitude is compounded by a plethora of scientific literature based on laboratory rodents—animals that are so dramatically different from their wild counterparts that extrapolation is virtually impossible (Stryjek and Pisula, 2008; Puckett et al., 2018). Additionally, decision makers may be apathetic regarding urban rodents owing to a perception that infestations and related harms are inevitable, and attempts to address them fruitless. Finally, a lack of substantive gains regarding urban rodent-related issues may be related to the fact that the responsibility for these issues is not easily assigned to any one sector or discipline. This has resulted in a siloed approach in which gains are made in specific areas (such as pest-control techniques), without moving the field of urban rodent research forward as a whole (Parsons et al., 2016).

We consider our special topics issue as a global "call to action" for researchers to help address these gaps and barriers through fresh, innovative, and multidisciplinary approaches. Therefore, in preparing this issue, we have brought together authors and reviewers from a wide array of field and laboratory-based disciplines (genetics, ecology, pest management, social sciences, public health) and from countries around the world (Australia, Brazil, Canada, Finland, France, Germany, Hungary, Japan, Netherlands, New Zealand, Poland, and the United States) to share as many ideas and perspectives as possible. From the resulting set of manuscripts, a number of critical themes emerged.

The research presented here paints a compelling picture of the complex interactions between rats and the urban environment. For example, Minter et al. show that within a specific neighborhood, the features of the urban built environment (i.e., variations in land use and building disrepair) did not significantly impact *Leptospira* spp. carriage in rats (*Rattus* spp.). However, variation in infection dynamics among cities suggested that broader features of the urban ecosystem impact pathogen ecology. Minter et al. found that although lethal rat control can produce a temporary decrease in the risk of *Leptospira* spp. infection in humans, only permanent and significant environmental modification was capable of producing a significant and sustained impact. The impact of the environment extends beyond *Leptospira* spp., with Cummings et al. showing that the distribution of newly identified influenza A virus in rats was significantly influenced by the urban microenvironment (e.g., parks vs. residential areas) and season.

Byers et al. present a review of rat movements in urban ecosystems, including how these movements are determined in equal measure by innate rat biology/behavior and by features of the specific environment in which rats reside, such as resource availability and anthropogenic barriers (e.g., roadways). Given that movement of rats can impact everything from the efficacy of rat control to the transmission of zoonotic disease among rats, this information may provide a lens through which to better understand the relationships between rodents and their city habitat. Going forward, it is clear that the study of disease ecology in urban rodents must include a detailed and thoughtful accounting of the role of the urban environment.

This issue also demonstrates the importance of understanding the potential impact of human intervention on rat ecology when seeking to monitor or mitigate rat-associated issues. Minter et al. showed that carriage of *Leptospria* spp. among rats is strongly influenced by specific social interactions (e.g., aggressive encounters) regardless of geographic location. This supports a growing body of evidence that interventions that upset established social structures and pathogen transmission pathways (e.g., indiscriminate lethal pest control) could have unpredictable consequences on public health risks. Richardson et al. showed that lethal control programs cause rapid and severe changes in rat population genetics—a human-driven evolution for which the fallout has never before been contemplated. Byers et al. showed that even the act of trapping rats is fraught with ecological complexities, as the probability of a rat entering a trap depends on the duration of the trapping campaign, as well as the demographics of the rats in the target population.

With regard to alternative strategies in rat control, the use of predator scents to repel rodents has been known for producing markedly different results in the laboratory as compared to the field. Using a comprehensive review, Bedoya-Pérez et al. show that key variables, such as habitat familiarity and resource availability dramatically impact the amount of risk that a rodent perceives, thus the efficacy of predator scents in rodent control will depend on the context in which they are presented.

Another emergent theme is the continuously changing face of rodent population and the pathogens they carry. This includes the first detection of influenza A virus in rats by Cummings et al. It also includes a description by Childs et al. of how the "transmission web" of established rodent associated zoonoses, such as Seoul Hantavirus and Lymphocytic Choriomeningitis Virus, has evolved over time and is likely to continue evolving. Kosoy and Bai expand on this theme by highlighting the fact that the health burden associated with rodent-associated pathogens, such as *Bartonella* spp., is likely to increase as a result of the intersection of urbanization and ongoing, related changes in urban rat, vector, and pathogen ecology.

Using a population genetics approach, Russell et al. found that there have been multiple introductions of *R. norvegicus* and *R. rattus* in New Zealand, as well as a continued spread of both rat species within some parts of the country. This work has important implications for our understanding of how rats and their pathogens could move across the landscape, and complements the work presented by Kosoy and Bai which shows how rat ecology, including rat movement,

has influenced the distribution and prevalence of different *Bartonella* spp. at scales ranging from a city block to the entire globe.

Innovation was also a feature of much of the research presented here. For example, Minter et al. present a novel mathematical model for identifying the optimum combination of control methods to prevent leptospirosis. Stryjek et al. have produced an innovative hanging trap that allows several parameters, such as rodent-type and distance from target to be preset. Because the trap is inconspicuous, it overcomes the tendency of rodents to avoid novel structures, such as conventional, ground-based traps (e.g., neophobia).

As often occurs during field-based research, serendipity, or discovering the unexpected, was key to Parsons et al. findings. Intending to study rat scents as possible deterrents, their study site was over-run by feral cats. Using a combination of microchipped rats and cameras deployed throughout a large study area, Parsons et al. were able to document, for the first time, the degree to which feral cats prey on rats, showing that cats are not likely to be an effective means of urban rat control. Murray et al. were able to use a novel partnership between researchers and private practitioners to investigate the degree to which rat complaints correlated with trapping data and can be used as a metric to monitor rat populations in the future.

In addition to presenting novel products and methodologies, several authors presented a number of compelling ideas and perspectives that can potentially be used to revolutionize urban rodent research in the future. For instance, Stephen astutely recognized a number of entrenched research paradigms that have hampered the progress of the field. Specifically, a largely reactionary viewpoint focused on documenting current or past hazards and adverse events, as well as the existence of a patchwork of disconnected approaches to urban rodent research and policy. This led to programs that are fragmented, inefficient, or even counterproductive. He suggests a paradigm shift toward the production of actionable intelligence, particularly regarding factors associated with vulnerability and resilience.

In order to understand the true complexity of the urban ecosystem, it is clear that a multidisciplinary and collaborative approach is needed: no one discipline or sector has the knowledge or capacity to manage on its own. A diversity of perspectives can be combined to provide a more comprehensive picture of the problem at hand. By reaching out across disciplinary lines into field and laboratory-based studies, it is possible to identify approaches that can be adapted and developed to foster true innovation. It is important to note, however, that this diversity of voices should not only include researchers, but also members of the public and decision makers, who ultimately determine the true impact of research findings (Stephen). The onus is therefore on urban rodent researchers, now and in the future, to venture beyond laboratories and trapping sites, and engage with those who will ultimately determine the relevance and value of what we do. This is perhaps the most important first step toward creating significant and meaningful changes in the trends regarding urban rodent monitoring and mitigation.

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MP, CH, MC, and CJ wrote and edited the article.

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REFERENCES

Aplin, K. P., Chesser, T., and ten Have, J. (2003). "Evolutionary biology of the genus Rattus: profile of an archetypal rodent pest," in *Rats, Mice and People: Rodent Biology and Management,* eds G. R. Singleton, L. A. Hinds, C. J. Krebs, and D. M. Spratt (Canberra, ACT: Australian Centre for International Agricultural Research Monograph), 487–98.

Banks, P. B., Bytheway, J. P., Carthey, A. J., Hughes, N. K., and Price, C. J. (2014). "Olfaction and predator-prey interactions amongst mammals in Australia," in *Carnivores of Australia: Past, Present and Future,* eds A. S. Glen and C. R. Dickman (Collingwood, VIC: CSIRO Publishing), 389.

Dyson, K., Ziter, C., Fuentes, T. L., and Patterson, M. S. (2019). Conducting urban ecology research on private property: advice for new urban ecologists. *J. Urban Ecol.* 5:juz001. doi: 10.1093/jue/juz001

Feng, A. Y., and Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosyst.* 17, 149–162. doi: 10.1007/s11252-013-0305-4

German, D., and Latkin, C. A. (2016). Exposure to urban rats as a community stressor among low-income urban residents. *J. Community Psychol.* 44, 249–262. doi: 10.1002/jcop.21762

Himsworth, C. G., Feng, A. Y., Parsons, K., Kerr, T., and Patrick, D. M. (2013). Using experiential knowledge to understand urban rat ecology: a survey of Canadian pest control professionals. *Urban Ecosyst.* 16, 341–350. doi: 10.1007/s11252-012-0261-4

Lam, R., Byers, K. A., and Himsworth, C. G. (2018). Beyond zoonosis: the mental health impacts of rat exposure on impoverished urban neighborhoods. *J. Environ. Health* 81, 8–11.

Lund, M. (1994). "Commensal rodents," in *Rodent Pests and Their Control,* eds A. P. Buckle and R. H. Smith (Oxfordshire: CAB International), 23–43.

Parsons, M. H., Banks, P. B., Deutsch, M. A., Corrigan, R. F., and Munshi-South, J. (2017). Trends in urban rat ecology: a framework to define the prevailing knowledge gaps and incentives for academia, pest management professionals (PMPs) and public health agencies to participate. *J. Urban Ecol.* 3:jux005. doi: 10.1093/jue/jux005

Parsons, M. H., Sarno, R., and Deutsch, M. (2015). Jump-starting urban rat research: conspecific pheromones recruit wild rats into a behavioral and pathogen-monitoring assay. *Front. Ecol. Evol.* 3:146. doi: 10.3389/fevo.2015.00146

Parsons, M. H., Sarno, R. J., and Deutsch, M. A. (2016). A detailed protocol to enable safe-handling, preemptive detection, and systematic surveillance of rat-vectored pathogens in the urban environment. *Front. Public Health* 4:132. doi: 10.3389/fpubh.2016.00132

Pimentel, D., Zuniga, R., and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52, 273–288. doi: 10.1016/j.ecolecon.2004.10.002

Puckett, E. E., Micci-Smith, O., and Munshi-South, J. (2018). Genomic analyses identify multiple Asian origins and deeply diverged mitochondrial clades in inbred brown rats (*Rattus norvegicus*). *Evol. Appl.* 11, 718–726. doi: 10.1111/eva.12572

Stryjek, R., and Pisula, W. (2008). Warsaw Wild Captive Pisula Stryjek rats (WWCPS)-establishing a breeding colony of Norway Rat in captivity. *Polish Psychol. Bull.* 39, 67–70. doi: 10.2478/v10059-008-0011-x

Traweger, D., Travnitzky, R., Moser, C., Walzer, C., and Bernatzky, G. (2006). Habitat preferences and distribution of the brown rat (Rattus norvegicusBerk.) in the city of Salzburg (Austria): implications for an urban rat management. *J. Pest Sci.* 79, 113–125. doi: 10.1007/s10340-006-0123-z

United Nations (2018). *68% of the World Population Projected to Live in Urban Areas by 2050, Says UN*. United Nations Department of Economic and Social Affairs. Available online at: https://www.un.org/development/desa/en/news/population/2018-revision-of-world-urbanization-prospects.html (accessed August 2, 2019).

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Evolving Urban Wildlife Health Surveillance to Intelligence for Pest Mitigation and Monitoring

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This paper introduces the concept of harm reduction-based health intelligence as the next step in the evolution of urban wildlife surveillance. There are three reasons to evolve urban wildlife health surveillance: (1) proactive steps to reduce vulnerability to health and safety impacts requires an understanding of environments and social structures as well as of the abundance and distribution of animals or hazards; (2) a hazard-by-hazard approach to surveillance causes management to be reactive rather than proactive; and (3) growing interest in urban wildlife ecology, conservation, and welfare plus the growing recognition of the value of urban wildlife for human well-being requires surveillance to be interested in protecting wildlife health as well as human health. Three strategies to help evolve urban wildlife surveillance to health intelligence are; (1) expand from only tracking a single species or a single threat to also tracking factors that increase the vulnerability of the pests and people in a shared urban setting; (2) be integrative and recognize that multiple concurrent harmful things are affecting people, pests and other species in their shared environments; and (3) develop new collaborative approaches to prevent or mitigate persistent harms from persistent pests without eliminating the pests. This article proposes that harm reduction-based intelligence will better equip city planners and pest managers to identify opportunities to act in advance of significant and concurrent harms to people, infrastructure, and wildlife.

Keywords: urban, wildlife, health intelligence, surveillance, harm reduction

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Our attitudes toward wild animals determine if we consider them a pest or as species to be tolerated and even conserved (Delibes-Mateos et al., 2011; Jones et al., 2012; Russell, 2014). For example, protecting rat welfare is of paramount importance in scientific research (Kaliste, 2004) yet our attitudes to urban rats are usually much less magnanimous. Rats that are free-ranging in undisturbed habitats, such as the rock-rat (*Zyzomys palatalis*) in Australia, are subjects of conservation efforts (Brook et al., 2002) whereas urban brown rats (*Rattus norvegicus*) are frequently the target of eradication efforts (Capizzi et al., 2014). In the world of urban wildlife health, a quick Google search will show most attention focuses on the role of rats as sources of hazards to other species, such as secondary poisoning of raptors or rodent-borne zoonotic infections. The literature rarely focuses on health for the inherent sake of the rat. Kirkwood and Sainsbury (1996) identified four factors that influence attitudes toward wildlife; (i) the extent to which we are responsible for harm to them; (ii) the extent to which the harmed animals are under our stewardship; (iii) the severity of the problem the wildlife face and (iv) cultural and economic factors, including the popularity of the species involved. The authors noted the illogical but heavily

weighted role popularity plays. It is, therefore, easy to understand why wildlife health surveillance rarely prioritizes efforts to protect and promote the health of urban wildlife, which we so often consider to be pests rather than wildlife.

Wildlife health surveillance has historically focused on generating early warning signals of risks to society or on tracking specific infectious diseases of concern for wildlife managers (Stephen and Duncan, 2017). There is a long history of wildlife serving as bio-sentinels for the effects and distribution of environmental pollutants and pathogens (Kuiken et al., 2005; Reif, 2011). Wildlife disease surveillance has been used as a guide to predict and prevent new zoonotic disease risks to the public (Stitt et al., 2007). Despite these successes, there is a growing dissatisfaction with the use of wildlife surveillance, largely in reaction to adverse events. Instead, there are new expectations to produce signals to protect wildlife and human health concurrently and proactively.

There are three reasons to reconsider how we design and use urban wildlife health surveillance. First, changes in urban wildlife social structures and habitats are known to determine wildlife vulnerability to environmental hazards (ex. Bradley and Altizer, 2007; Lee et al., 2018). Tracking clues of changing vulnerability may allow more targeted and proactive actions to avoid impacts on public health and safety as well as to protect wildlife health. Managers may be better able to take precautionary steps when vulnerability is tracked, as opposed to focussing only on the abundance and distribution of animals or hazards. Second, a hazard-by-hazard approach to risk management is insufficient to reduce vulnerability and promote resilience to emerging risks. It dooms management strategies to remain reactive rather than proactive. In an era of unprecedented social, landscape, and climate changes, emerging risks are the norm, reducing the usefulness of surveillance that only tracks known hazards (Stephen et al., 2015). Third, growing interest in urban wildlife ecology, conservation, and welfare (ex. Adams, 2005) and growing recognition of the value of urban wildlife areas and biodiversity for human well-being (Soulsbury and White, 2016) suggest that better information is needed to reciprocally promote the health of wildlife and people in urban environments. Protection of the health of one species should not come at an unsustainable or unacceptable expense to another. Shifting the target of attention away from threats and hazards to health and vulnerability may allow for earlier interventions and open more options for surveillance and action.

Wildlife health is increasingly being viewed as the cumulative effect of social, physical, and biological influences on the capacity of individuals and populations to; (i) access their needs for daily living; (ii) have capacity to cope with and adapt to stressors and change and (iii) meet ecological and social expectations (Nordenfelt, 2011; Hanisch et al., 2012; Stephen, 2014). Wildlife health programs rarely assembled all three aspects into a complete picture. As such, control programs tend to be fragmented and inefficient, or worse, unintentionally impede each other. For example, it has been proposed that rodent control programs focussed on lethal population reduction may increase the prevalence of zoonotic pathogens in some urban rat populations (Lee et al., 2018). Wildlife health surveillance usually does not track factors influencing population vulnerability, leaving that instead to population ecologists who in turn do not take full advantage of health surveillance information.

A significant impediment to wildlife surveillance is the lack of agreement on the indicator threshold that signals the need for an intervention. It is generally accepted that human endeavors should not unnecessarily compromise wild animal welfare (Kirkwood and Sainsbury, 1996), but the threshold for "unnecessary compromise" is ill-defined. It is also unclear if that threshold is different for urban and non-urban wildlife. For example, some stakeholders might have zero risk tolerance and demand eradication of infections from an urban wild population while others see an animal's microbiological flora as part of our biodiversity heritage and thus their associated risks better controlled by managing the human dimensions of risk. First principles of population health (ex. Karpati et al., 2002; Gowan et al., 2014) argue for surveillance that focuses on the determinants of health and vulnerability rather than only on the presence and distribution of hazards, but knowledge gaps preclude evidence-based recommendations on the best variables to monitor in urban wildlife. Moreover, divergent social values complicate finding consensus on thresholds that meet varying risk perceptions.

There are three strategies to evolving wildlife health surveillance to meet the changing landscape of urban wildlife risk to both address growing concerns about the equitable protection of human and urban wildlife health, and to provide more integrated signals to support a precautionary, rather than reactionary, urban pest management approach. First, surveillance needs to evolve from tracking a single threat to an intelligence system that not only tracks hazards and adverse outcomes but also tracks factors that increase the vulnerability of the pests and people in a shared urban setting. For example, the reasons for failure or success of wildlife health management are usually social, rather than biological or physical factors. Therefore, an urban wildlife health intelligence system needs to be able to characterize changes in human dimensions of risk (including changes to the built environment), social conditions affecting human exposure or susceptibility to wildlife-associated harms, and changes in risk perception. At the same time attention must be paid to variables that create cumulative stressors on urban wildlife that increase their vulnerability to new hazards. Research will be needed to specify and prioritize determinants of vulnerability for a given location. However, a general wildlife health intelligence system needs to be able to gather information on; (i) the biological endowment of the population (ex diseases and stressors); (ii) the animal's social environment (ex. extent of competition and demographics); (iii) the quality and abundance of the needs for daily living (ex. food supplies and habitat availability); (iv) their abiotic environment (ex. climate variables such as floods); (vi) sources of direct mortality (ex. lethal pest control), and (vii) changing human expectations (ex. social attitude and municipal policies). This strategy is compatible with ecological-based pest management (Singleton et al., 1999) as well as bio-economic approaches to rodent pest control (Stenseth et al., 2003). It is also compatible with opinions that ecological-based pest management must attend to social and

cultural dimensions to ensure pest control efforts are adopted and sustained (Palis et al., 2007).

Second, an urban wildlife health intelligence system needs to recognize that multiple concurrent harms are affecting people, pests, and other species in their shared environments. Over abundant rodents, for example, can cause various social harms (ex fear, zoonotic disease transmissions and damage to housing infrastructure); harm rodent predators and scavengers (ex. impacts of rodenticides on the safety and availability of prey) and impact the rodents themselves (ex. reduced welfare through intra-specific competition and increased non-zoonotic rodent diseases). The importance of each harm will vary between stakeholders, but it is inevitable that their interconnections and interdependencies will create unique challenges and opportunities for intervention. Recognizing the ties between society, nature, and technology has been proposed as a better way to create a comprehensive, cohesive pest management strategy that is ecologically sound and socially acceptable (Mougenot and Roussel, 2005).

Third, given that cities can find it very difficult to eliminate their urban wildlife problems on a sustainable basis (Fernández et al., 2007; Himsworth et al., 2013), new collaborative approaches are needed to find interventions that can prevent or mitigate persistent harms from persistent pests without eliminating the pest entirely.

The various harms created by urban wildlife are embedded in complex socio-ecological systems. Therefore, multiple points of view are needed to characterize and respond to urban pest problems. Because underlying, incessant social conditions can cause pest problems to persist, there is often a feeling of helplessness in efforts to eliminate their negative impacts. As we move from individual animals to the population and ecosystem level, risk management decision-making becomes more variable, uncertain, and complex. The inseparable links between the individual, social and ecologic levels of harm from urban wildlife suggest that multi-level interventions are required to make increment gains in the health of wildlife, human communities and their shared ecosystems. Harm reduction is a set of perspectives and processes that might offer insights into how to evolve urban wildlife health surveillance to this end.

Harm reduction is most often used to describe a set of public health strategies to reduce the harmful consequences of addictive behaviors on individuals and society (Hunt et al., 2003). It acknowledges that society is unlikely to eliminate substances like illicit drugs and that attempts at elimination have been insufficient to prevent the harms arising from addiction. Like illicit drugs, urban pests and their associated hazards are often persistent problems that are hard to eradicate. Harm reduction programs aim to prevent or reduce adverse consequences to all community members rather than only targeting the hazardous substance or circumstance. Harm reduction applies to all the determinants of health and not merely problematic risks. It involves pragmatic approaches to remove barriers to implementing knowledge to protect health and promote sustainability. Harm reduction aims to decrease the impact on multiple actors in a community (in this case human and animals) by addressing both the amount of harm and its impacts (Marlatt, 1996). The amount of harm can be reduced by reducing exposure to and sensitivity of a population to hazards whereas the impacts are addressed by promoting populations' capacity to cope with the harm and by addressing harms as cumulative effects (Stephen et al., 2018b).

An urban wildlife health intelligence program based on harm reduction would need to adopt the following six principles (based on BCMOH[1]). First, collect data on the nature and distribution of the social, health, and animal harms as opposed to only tracking the causes of harms such as pathogens or diseases. Second, recognize that pest or their diseases are not likely to be eliminated in the short term and therefore seek information on variables that can pragmatically be manipulated to reduce animal and human vulnerability to pest related harms. Third, develop an information network that will reveal feasible options to help populations cope with existing harms within the current circumstances while efforts to eliminate these harms are ongoing. Fourth, health intelligence signals need to identify and prioritize actions that can produce incremental gains that can be built on over time. Fifth, recognize that actions attacking one harm may have unintended consequences for other harms, necessitating integrated analysis. Finally, health intelligence systems need to be people oriented and actively engage a diversity of players to find pathways and control points that can be targeted to reduce harms across various perspectives, priorities, and values.

Figure 1 illustrates the relationship between health intelligence and harm reduction. Health intelligence helps to set priorities for harm reduction actions. Health intelligence uses surveillance to create situational awareness of harmful substances or circumstances along with reconnaissance to characterize the local conditions that may impede or enable interventions. The total amount of harm caused by urban wildlife is affected by the total harms across social and animal domains and the impacts of those harms. A socio-ecological assessment of harms helps identify targets for health intelligence.

Harm reduction place decisions makers' needs at the forefront of their design and implementation (Regmi et al., 2016). As there are multiple layers of decision makers in pest management, from the household to senior government officials, a harm reduction-based health intelligence system needs a good human network to ensure analysts understand the information needs, priorities, and thresholds across the decision-making spectrum. Contextual understanding helps turn information into intelligence and increases the likelihood that surveillance and reconnaissance results will be put into action (Haines et al., 2004). As much attention needs to be placed on human intelligence and information networks as on datasets to turn diverse sets of information into coherent intelligence outputs.

Harm reduction-based health intelligence should help people make informed decisions and empower them to minimize harms by identifying ways to reduce negative impacts until a hazard can be moderated or eliminated. Harm reduction

[1]British Columbia Ministry of Health (BCMOH). *Harm Reduction: a British Columbia Community Guide. British Columbia Ministry of Health.* Available online at: http://www.health.gov.bc.ca/library/publications/year/2005/hrcommunityguide.pdf (Accessed May 5, 2018).

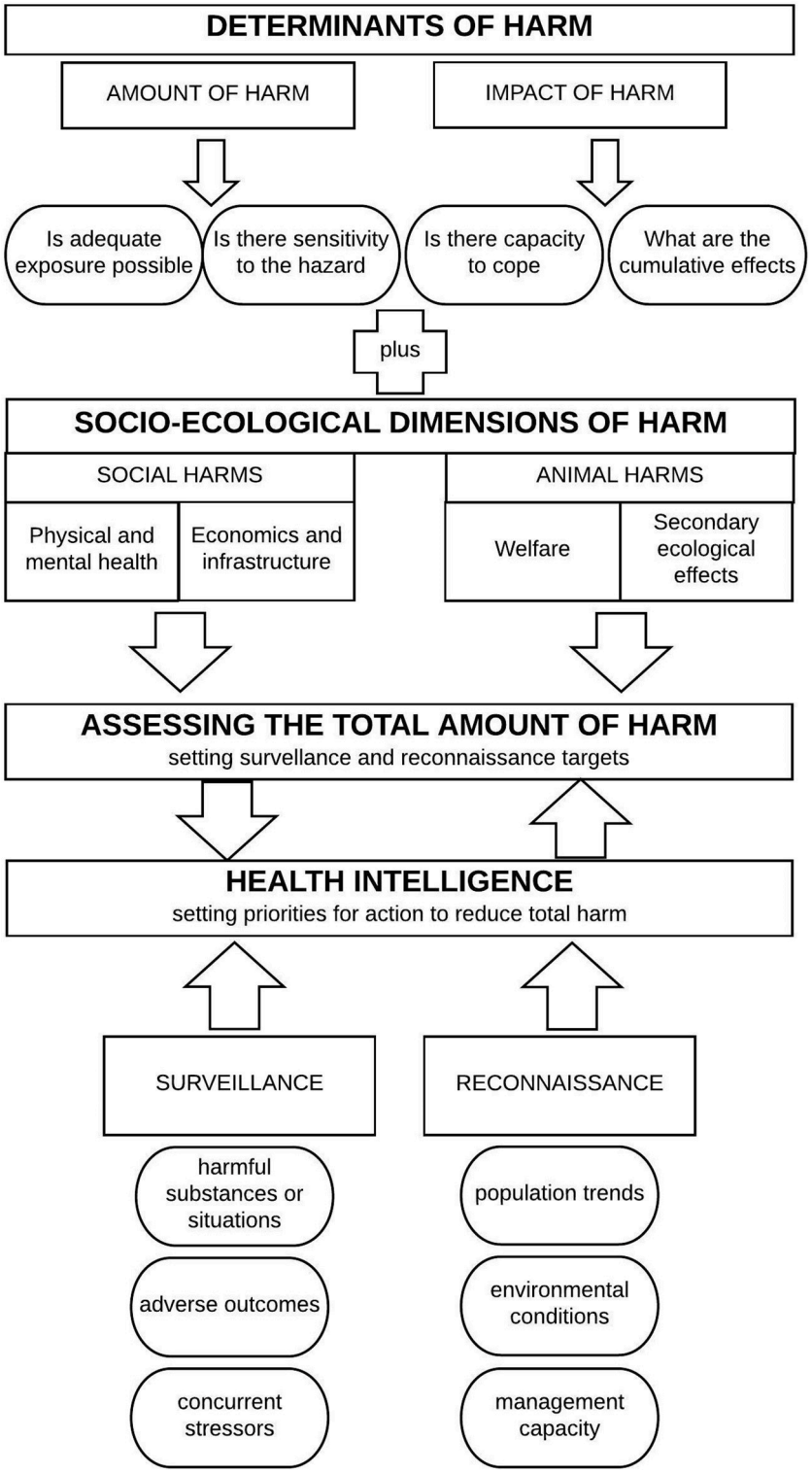

FIGURE 1 | Conceptual model of the integration of health intelligence and harm reduction to set priorities for urban wildlife health surveillance and action.

promotes collaborative policy and action by discovering means for horizontal, cooperative approaches to protecting health in advance of serious, irreversible impacts. Adopting a harm reduction perspective is not a rejection of the current surveillance paradigm but rather a call to expand our scope of observations to ensure that opportunities to lessen harms can be identified

and acted on while we strive to directly prevent or avoid the negative consequences of urban wildlife. Decision-makers need to understand the full scope of a pest problem and how to; (i) recognize priority problems and needs, (ii) track progress to evaluate the impact of interventions, and (iii) make evidence-based decisions on policy, program design and resource allocation (Regmi et al., 2016).

It is important to note the word "evolving" in the title of this paper. The scope of most wildlife health programs is more limited than what I am proposing above. Neither literature nor legislation describe the necessary features of an urban wildlife health intelligence system. Stephen et al. (2018a) have proposed a generic set of attributes of national wildlife health surveillance programs that share features with those described in this paper particularly; (i) the expectation to integrate health into risk management planning and assessment, (ii) an interest in the determinants of health as well as health outcomes, and (iii) the need to engage players outside of traditional wildlife health sectors. But their recommendations emphasized free-ranging non-urban wildlife which are more often of concern to trade and conservation. An urban wildlife health intelligence approach needs local information and context to identify feasible and acceptable strategies to reduce pest-associated harms. It is often beyond the capacity of departments responsible for local pest control to have the expertise and human resources to gather all the necessary information by themselves. Strategic partners to enable collective intelligence across multiple departments such as public health, urban planning, and sanitation would be needed. Investment should focus on centralized capacity to collect, integrate, and assess data already being gathered by other departments, supplemented with scientific literature and insights gathered through participatory processes.

Working examples of an urban health intelligence system do not yet exist in the literature but examples exist in other sectors. Epidemic intelligence and public health observatories are public health approaches that use an intelligence approach (Hemmings and Wilkinson, 2003; Paquet et al., 2006). Both track multiple lines of information on hazards, hosts and environments to identify vulnerabilities and threats before they impact populations. The Canadian Wildlife Health Cooperative has adopted these concepts to evolve its surveillance database to an intelligence platform. Its ongoing work is exploring how ecological, environmental, and microbiological clues from nature and farms can inform wild bird avian influenza preparedness. This article call for an urban wildlife health intelligence system is a response to the growing need for tools and practices to link diverse data sources to better reflect the origins of and solutions to problems from human-wildlife interactions. However, specifying the precise information to track will depend, in part, on the species being managed, the availability of contextual information and emerging research to identify the most reliable signals. The intent of this paper is to inspire investigation and investment to move this idea from the conception to implementation.

There will always be a need to control urban wildlife. As we become more urbanized, there will also be the need to sustain the benefits of urban wildlife for human well-being. The world is experiencing unprecedented rates of social and environmental changes due to climate change, global movement of people and products, and rapid urbanization (Biermann, 2007; Horton et al., 2014). A new approach is needed to provide the necessary situational awareness to stay on top of the inevitable emerging risks that will arise with these changes. The concept of harm reduction and the needs to expand the realms of wildlife surveillance have been recommended elsewhere (ex. Stephen and Duncan, 2017; Stephen et al., 2018b) and serve as a basis to advocate for the changes discussed in this article. By evolving urban wildlife health surveillance from its tradition of solely looking at the distribution and prevalence of hazards to a harm reduction-based intelligence approach, I propose that city planners and pest managers will be better equipped to identify opportunities and needs to act in advance of significant harms to people, infrastructure and the wildlife that call our cities home.

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REFERENCES

Adams, L. W. (2005). Urban wildlife ecology and conservation: a brief history of the discipline. *Urban Ecosyst.* 8, 139–156. doi: 10.1007/s11252-005-4377-7

Biermann, F. (2007). 'Earth system governance' as a crosscutting theme of global change research. *Global Environ. Change* 17, 326–337. doi: 10.1016/j.gloenvcha.2006.11.010

Bradley, C. A., and Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends Ecol. Evol.* 22, 95–102. doi: 10.1016/j.tree.2006.11.001

Brook, B. W., Griffiths, A. D., and Puckey, H. L. (2002). Modelling strategies for the management of the critically endangered Carpentarian rock-rat (*Zyzomys palatalis*) of northern Australia. *J. Environ. Manage.* 65, 355–368. doi: 10.1016/S0301-4797(02)90561-X

Capizzi, D., Bertolino, S., and Mortelliti, A. (2014). Rating the rat: global patterns and research priorities in impacts and management of rodent pests. *Mamm. Rev.* 44, 148–162. doi: 10.1111/mam.12019

Delibes-Mateos, M., Smith, A. T., Slobodchikoff, C. N., and Swenson, J. E. (2011). The paradox of keystone species persecuted as pests: a call for the conservation of abundant small mammals in their native range. *Biol. Conserv.* 144, 1335–1346. doi: 10.1016/j.biocon.2011.02.012

Fernández, M. S., Cavia, R., Cueto, G. R., and Suárez, O. V. (2007). Implementation and evaluation of an integrated program for rodent control in a shantytown of Buenos Aires City, Argentina. *EcoHealth* 4, 271–277. doi: 10.1007/s10393-007-0122-4

Gowan, M. E., Kirk, R. C., and Sloan, J. A. (2014). Building resiliency: a cross-sectional study examining relationships among health-related quality of life, well-being, and disaster preparedness. *Health Q. Life Out.* 12:85 doi: 10.1186/1477-7525-12-85

Haines, A., Kuruvilla, S., and Borchert, M. (2004). Bridging the implementation gap between knowledge and action for health. *Bull. World Health Organ.* 82, 724–731.

Hanisch, S., Riley, S., and Nelson, M. (2012). Promoting wildlife health or fighting wildlife disease: insights from history, philosophy and science. *Wildl. Soc. Bull.* 36, 477–482. doi: 10.1002/wsb.163

Hemmings, J., and Wilkinson, J. (2003). "What is a public health observatory?" *J. Epidemiol. Commun. Health* 57, 324–326. doi: 10.1136/jech.57.5.324

Himsworth, C. G., Feng, A. Y., Parsons, K., Kerr, T., and Patrick, D. M. (2013). Using experiential knowledge to understand urban rat ecology: a survey of Canadian pest control professionals. *Urban Ecosyst*. 16, 341–350. doi: 10.1007/s11252-012-0261-4

Horton, R., Beaglehole, R., Bonita, R., Raeburn, J., McKee, M., and Wall, S. (2014). From public to planetary health: a manifesto. *Lancet* 383:847. doi: 10.1016/S0140-6736(14)60409-8

Hunt, N., Ashton, M., Lenton, S., Mitcheson, L., Nelles, B., and Stimson, G. (2003). *A Review of the Evidence-Base for Harm Reduction Approaches to Drug Use*. London: Forward Thinking on Drugs.

Jones, C., Barron, M., Warburton, B., Coleman, M., Lyver, P. O. B., Nugent, G. (2012). Serving two masters: reconciling economic and biodiversity outcomes of brushtail possum *(Trichosurus vulpecula)* fur harvest in an indigenous New Zealand forest. *Biol. Conserv.* 153, 143–152. doi: 10.1016/j.biocon.2012.04.016

Kaliste, E. (2004). *The Welfare of Laboratory Animals*, Vol. 2. Dordrecht: Springer Science and Business Media.

Karpati, A., Galea, S., Awerbuch, T., and Levins, R. (2002). Variability and vulnerability at the ecological level: implications for understanding the social determinants of health. *Am. J. Public Health* 92, 1768–1772. doi: 10.2105/AJPH.92.11.1768

Kirkwood, J. K., and Sainsbury, A. W. (1996). Ethics of interventions for the welfare of free-living wild animals. *Anim. Welfare* 5, 235–244.

Kuiken, T., Leighton, F. A., Fouchier, R. A., LeDuc, J. W., Peiris, J. S., and Schudel, A., et al. (2005). Public health. Pathogen surveillance in animals. *Science* 309, 1680–1681 doi: 10.1126/science.1113310

Lee, M. J., Byers, K. A., Donovan, C. M., Bidulka, J. J., Stephen, C., Patrick, D. M., et al. (2018). Effects of culling on *Leptospira interrogans* carriage by rats. *Emerg. Infect. Dis*. 24, 356–360. doi: 10.3201/eid2402.171371

Marlatt, G. A. (1996). Harm reduction: come as you are. *Addict. Behav*. 21, 779–788. doi: 10.1016/0306-4603(96)00042-1

Mougenot, C., and Roussel, L. (2005). To poison or to trap? The ecologisation of 'pest'control. *Soc. Ruralis* 45, 115–129. doi: 10.1111/j.1467-9523.2005.00294.x

Nordenfelt, L. (2011). Health and welfare in animals and humans. *Acta Biotheora.* 59, 139–152. doi: 10.1007/s10441-011-9125-1

Palis, F. G., Singleton, G., Sumalde, Z., and Hossain, M. (2007). Social and cultural dimensions of rodent pest management. *Integr. Zool.* 2, 174–183. doi: 10.1111/j.1749-4877.2007.00057.x

Paquet, C., Coulombier, D., Kaiser, R., and Ciotti, M. (2006). Epidemic intelligence: a new framework for strengthening disease surveillance in Europe. *Euro Surveill.* 11, 212–214. doi: 10.2807/esm.11.12.00665-en

Regmi, K., Bendel, N., and Gee, I. (2016). "Public health intelligence: an overview," in *Public Health Intelligence,* eds K. Regmi and I. Gee (New York, NY: Springer International Publishing), 1–18.

Reif, J. S. (2011). Animal sentinels for environmental and public health. *Public Health Rep*. 126 (Suppl 1), 50–57. doi: 10.1177/00333549111260S108

Russell, J. C. (2014). A comparison of attitudes towards introduced wildlife in New Zealand in 1994 and 2012. *J. R. Soc. N. Z.* 44, 136–151. doi: 10.1080/03036758.2014.944192

Singleton, G. R., Leirs, H., Hinds, L. A., and Zhang, Z. (1999). *Ecologically-Based Management of Rodent Pests–Re-Evaluating Our Approach to an Old Problem*. Canberra: Australian Centre for International Agricultural Research (ACIAR), 17–29.

Soulsbury, C. D., and White, P. C. (2016). Human–wildlife interactions in urban areas: a review of conflicts, benefits and opportunities. *Wildl. Res*. 42, 541–553. doi: 10.1071/WR14229

Stenseth, N. C., Leirs, H., Skonhoft, A., Davis, S. A., Pech, R. P., Andreassen, H. P., et al. (2003). Mice, rats, and people: the bio-economics of agricultural rodent pests. *Front. Ecol. Environ*. 1, 367–375. doi: 10.1890/1540-9295(2003)001%5b0367:MRAPTB%5d2.0.CO;2

Stephen, C. (2014). Toward a modernized definition of wildlife health. *J. Wildl. Dis*. 50, 427–430. doi: 10.7589/2013-11-305

Stephen, C., Berezowski, J., and Misra, V. (2015). Surprise is a neglected aspect of emerging infectious disease. *Ecohealth* 12, 208–211. doi: 10.1007/s10393-014-1001-4

Stephen, C., and Duncan, C. (2017). Can wildlife surveillance contribute to public health preparedness for climate change? A Canadian perspective. *Clim. Change* 141, 259–271. doi: 10.1007/s10584-016-1892-x

Stephen, C., Sleeman, J., Nguyen, N., Zimmer, P., Duff, J. P., Gavier-Widén, D., et al. (2018a). Proposed attributes of national wildlife health programmes. *Rev. Sci. Tech.* 37.

Stephen, C., Wittrock, J., and Wade, J. (2018b). Using a harm reduction approach in an environmental case study of fish and wildlife health. *Ecohealth* 15, 4–7. doi: 10.1007/s10393-017-1311-4

Stitt, T., Mountifield, J., and Stephen, C. (2007). Opportunities and obstacles to collecting wildlife disease data for public health purposes: results of a pilot study on Vancouver Island, British Columbia. *Can. Vet. J.* 48, 83–90.

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Temporal and Space-Use Changes by Rats in Response to Predation by Feral Cats in an Urban Ecosystem

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Feral cats (*Felis catus*) are predators that cause widespread loss of native wildlife in urban ecosystems. Despite these risks, cats are commonly released as control agents for city rats (*Rattus* spp.). Cats can influence their prey directly by killing or indirectly through changes to feeding or space-use. However, cats prefer defenseless prey, and there are no data suggesting that cats influence large (>300 g) urban rats. We used a pre-existing radiofrequency identification assay (microchipped rats and field cameras) and ethograms to assess the impact of cats, including temporal and space use patterns, on an active rat colony. From Dec 27, 2017 through May 28, 2018 we captured 306 videos of pre-identified cats and/or rats that shared the same space. There were three instances of predation and 20 stalking events. Logistic regression showed the likelihood of a rat being seen on a particular day is associated with the number of cats seen on the same day (OR = 0.1, $p < 0.001$) or previous day (OR = 0.15, $p < 0.001$). Space-use was also impacted. For every additional cat sighting, a rat is 1.19 times more likely to move in the direction of shelter. Our findings of low levels of predation support why ecologists believe the risks to native wildlife outweighs any benefits of releasing cats. Even though rats were less likely to be seen, they simply shifted their movements and remained present in the system. Our findings that cat presence led to fewer rat sightings may explain the common perception of their value as rat-predators despite the associated risks.

Keywords: city rats, *Felis catus*, New York city rat, Norway rats, *Rattus norvegicus*, urban wildlife interactions

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Pussycat, pussycat, where have you been?
I've been to London to visit the Queen.
Pussycat, pussycat, what did you there?
I frightened a little mouse under her chair.

… James W. Elliot (1870)

INTRODUCTION

Cat predation on rodents, particularly mice (*Mus*, *Peromyscus* spp.), is among the most recognized models of predator: prey interactions, inspiring nursery rhymes, cartoons and public perceptions. Many non-trained observers, however, cannot tell the difference between mice which weigh 20–35 g (Zielinski et al., 1992) and city rats which often weigh 10 times as much (Parsons et al., 2017; Combs et al., 2018). This misconception may be partly responsible for cats being widely considered

as a "natural" control tool for rats. However, predators have an energy budget, whereby their caloric intake is maximized (Jeschke et al., 2002) against potential risks from large or defensive prey (Embar et al., 2014). And thus, cats may be more likely to deplete birds and easier meals (e.g., smaller mammals and their young) before moving to rats. Further, as domesticated animals, cats are well adapted to survive on handouts from people (Montague et al., 2014). The domestication of cats, and abundance of easier to obtain alternative foods cast doubts on cats' inclination to prey on city rats and suppress their numbers.

Feral cats can impact on the dynamics of their prey, but there is much stronger data indicating that cats influence native wildlife (Kauhala et al., 2015; Kikillus et al., 2017) than city rats. For example, in Australia (Davies et al., 2017) and the US (Loss et al., 2013), cats represent the greatest source of anthropogenic mortality for native birds and mammals. This has been historically recognized on island ecosystems where cats are evolutionarily novel (Woinarski et al., 2017), but is also true for some continental (Loss and Marra, 2017) and urban systems (Pillay et al., 2018). At current rates of human urbanization, the latter are the fastest growing ecosystems in the world, with more than 70% of people expected to live in cities by 2050 (Zhou et al., 2013). In contrast to the native vertebrates most impacted by cats, city rats have coexisted with cats for centuries. These rats are well adapted to urban ecosystems; they represent a large prey item for all but the boldest cats and can defend themselves. Yet some densely populated cities such as Chicago, IL., USA are widely reported in the media (Glanton, 2017) as making mass efforts to use cats as rat control tools. Not only is releasing cats a risk for wildlife, but it also invites welfare concerns for the cats themselves. For instance, it is common knowledge that a well-fed and cared for cat may coexist peacefully alongside rats. Thus, those who employ cats as pest-control solutions may intentionally avoid feeding them in order to prompt them to hunt (MP, pers. obs.). More conclusive evidence is necessary before cats can be justified as control instruments for rats.

Because of their larger size, city rats may be less vulnerable to cats than native rodents. Feral cats tend to prefer smaller prey, including smaller rodents with a <250 g threshold (Childs, 1986). Much of the evidence identifying cat impacts on *Rattus* species is from islands, where insular dwarfism may lead to animals be smaller than continental conspecifics (e.g., Foster's rule, Rozzi and Lomolino, 2017). For instance, Karl and Best (1982) examined 229 scats from feral cats on Stewart Island, New Zealand. Rats (*Rattus* spp.) occurred in 93% of the scats (as compared to 44% birds). However, adult rats in these systems tends to be around 150 g (or half the size of a New York City rat; Parsons et al., 2017; Combs et al., 2018), including Polynesian rats (*Rattus exulans*) which are the smallest of the human-commensal *Rattus*. Similarly, Fitzgerald et al. (1991) examined 8 years of feral cat scats on Raoul Island. Ninety percent of the rats were Polynesian rats, with less than 10% from the larger (though still smaller than occurs on mainland), Norway rat (*R. norvegicus*).

Among the few continental studies that examined the influence of cats on rats, there were mixed results. In Finland, researchers found that 72% of all prey brought home by cats were rodents. However the authors did not distinguish between mice and rats, and indicated that almost half of all kills belonged to exceptionally large cats (Kauhala et al., 2015). Experimental release of 20 native long-haired rats (*Rattus villosissimus*) in Australia led to rapid extirpation by cats (Frank et al., 2014). However, these rats were also about half the size (150 g) of most city rats. Also in Australia, the analysis of stomach contents from 80 feral cats revealed 80% of their diet was from rodents, but the majority of rats were identified as long-haired rats (Yip et al., 2015).

There is no evidence that cats can directly suppress populations of city rats. However, predation impacts can also arise indirectly via non-lethal effects on prey from the risks of predation. The scent from cats (*Felus catus*) alone is enough to lower reproductive capacity in some mice (Kelliher and Wersinger, 2009; Voznessenskaya, 2014) and rats (Naidenko et al., 2003; Voznessenskaya et al., 2003; Voznessenskaya, 2014). Predation risk can also limit access to food (Herman and Valone, 2000), an effect that could be exaggerated in urban systems. In the urban environment, rats must navigate exposed environments when seeking new harborage or accessing food or water by some distance (unlike mice that can metabolically produce their own water; Schmidt-Nielsen and O'Dell, 1961, rats may drink up to 30 ml water/day, Siegel and Stuckey, 1947). Avoidance of exposed areas is common in many species of smaller, "vulnerable," animals (Apfelbach et al., 2015), from small marsupials in Tasmania (Parsons and Blumstein, 2010) to oldfield mice (*Peromyscus polionotus*) in Southern California (Orrock et al., 2004; Orrock and Danielson, 2009). It is thought the risk of predation drives fear of open spaces or full moons, where exposed, and forage less frequently or for shorter durations and remain closer to home (Orrock et al., 2004; Orrock and Danielson, 2009). However, evidence on the indirect effects from cats on city rats is also limited. Mixed predator scents from dogs and cats together, discouraged rodents from feeding trays, though the authors did not differentiate between mice (*Mastomys natalensis*) and ship rats (*R. rattus*) (Themb'alilahlwa et al., 2017). More recently, Carthey and Banks (2018) showed that free living ship rats recognized cat odor, but showed no anti-predator responses either in vigilance or feeding behaviors, suggesting that rats took the risks to access resources.

Indeed, despite the prominent knowledge gaps that persist regarding city rats, such as the well described influence that pheromones have to influence the behavior and physiology of rodents (Hurst and Beynon, 2004; Takács et al., 2016), almost no research has been performed *in situ* in the urban ecosystem (Parsons et al., 2017). This is because, if rats appear in the city, they exist on property owned by someone. The owners are not eager to allow experimentation by researchers unless the research leads to extermination. Thus, researchers have not had any means of studying urban rats in their natural habitat such as the Frank et al. (2014) experiment, and thereby providing stronger evidence for the influence of cats at a given location, either directly or indirectly, on city rats.

OBJECTIVES

In this paper we investigate how feral cats directly or indirectly impact urban rats. In our longitudinal rat research program in New York City, USA, we have access to an active rat colony inside a waste management facility where, via a pre-existing radio frequency identification (RFID) study, we live-trap, microchip, and release animals while studying their individual life histories, population demographics and behaviors (Parsons et al., 2017). Additionally, there are nearly two dozen feral cats that have lived in close proximity to this research site for several years. Specifically, we assessed whether variations in the number of cats present on a given day, or preceding day, would influence the number of rats observed, or whether the number of rats or humans would be a predictor of the number of cats seen. Our secondary interests were to determine whether the presence of cats had any effect on the prevalence of 8 common rat behaviors or direction of movement across a frequently utilized runway of the main colony.

METHODS

Study Area

New York City (NYC), 40.71° N, 74.01° W, is the second most populous city in North America at roughly 2,000 people/square mile (Griffith and Wong, 2007). With ∼8.5 million inhabitants, it consists of 5 boroughs located between the Hudson river and Atlantic Ocean. The climate has warm, moist summers (summer monthly highs average 27.8°C with 19.3°C minima) and cold winters (average monthly max is 5.0°C and −1.5°C minima) with an annual precipitation of 50–200 cm (NOAA). Brown rats (*Rattus norvegicus*) arrived from Europe by ship between 1700 and 1750 (Puckett et al., 2016; Combs et al., 2018). Because they had few natural predators, they rapidly reached pest status. Predators include red tailed hawks (*Buteo jamaicensis*), coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*) domestic dogs (especially terriers; *Canis familiaris*), mustellids such as the fisher (*Pekania pennanti*) and escaped pet ferrets (*Mustela putorius furo*) and feral cats (*Felis domesticus*). This area has been referred to as one of the "rattiest cities" on Earth (Robert Corrigan, Department Health and Mental Hygiene, NYC, ret.). This colloquial designation is the result of abundant wastes left out overnight, the amount of rubbish left outdoors and in parks, and the relative lack of predators.

Study Site

We received permission to utilize an industrial waste recycling site in Brooklyn, a south-eastern borough of NYC. The study site is located on one of the properties owned by Waste Management (WM). WM is a provider of comprehensive waste management services in North America, providing services that range from collection and disposal to recycling and renewable energy generation. As part of their commitment of *"supporting customers and communities,"* WM has agreed to host the New York City rat research project at Fordham University. The recycling plant is an indoor, semi-enclosed building that, as in any industrial, disturbed area, provides ample shelter for rodents.

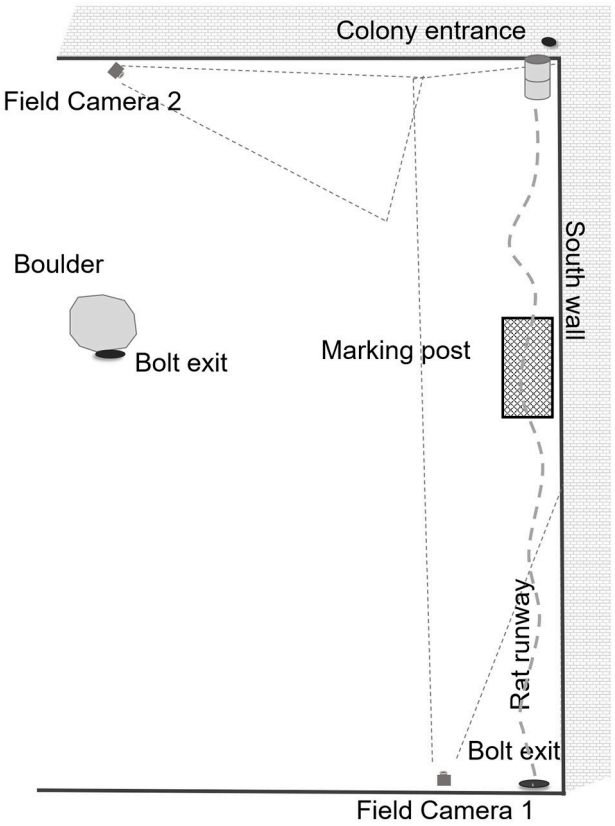

FIGURE 1 | Experimental set-up for feral cat trials at a semi-enclosed industrial building in New York City from Dec. 27, 2017—May 28, 2018.

Operations are ongoing day and evening, it is noisy from trucks and excavators and may be noxious from collected rubbish. There is a continuous supply of potential food for small animals, as central operations require that rubbish be brought in, sorted, recycled and disposed of, or shipped out. The site is not climate-controlled and animals may burrow under floors and deep within the walls, thus temperatures inside are variable depending on location. Our primary rat colony (**Figure 1**) is in a mostly unlit area that is dimly lit or dark day and night. The primary food supply is located immediately to the west of the main burrows.

Experimental Design

We have an ongoing rat research program at multiple sites in NYC, assessing rat behaviors in relation to scents, while producing ethograms and activity budgets to document city rat behaviors in the natural environment. During the latter part of our previous trials (Parsons et al. unpublished data), several cats entered our research area and persisted throughout the study. Instead of halting experiments, we designed an investigation to quantify the influence of cats on the rat behaviors and movements.

Our methods of live-capture and anesthetization have previously been reported (Parsons et al., 2015, 2016; Parsons et al., unpublished data). An RFID central processor with data logger (PADAR; UID, Chicago, IL, USA) had also been

permanently installed (Parsons et al., 2016). The rat colony (**Figure 1**) had an estimated 120–150 rats, based on 37 live captures (the majority of which were used in another study (Parsons et al., unpublished data). The average weight was 337 ± 13.6 g with average length of 375 ± 4.2 mm (including tail). Additionally, animals from this population are active during most of the day and evening, with some individuals actively recording their presence at RFID sensors the same day and night.

We deployed two infrared field cameras (Browning Strike Force Elite HD) with a 0.4 s trigger-speed, aimed from Northeast to Southeast and West to East across an active, well-established, rat colony inside the urban industrial center (**Figure 1**). We recorded these data from December 29, 2017 when the first cats were identified until May 28, 2018. We counted the number of triggers by cats, rats and people, and converted these to percent of total videos captured as our primary response variables, while using day as our observation period. We only recorded intervals where at least one rat or cat was captured in order to account for false triggers by researchers, wind, and birds on days when there was otherwise no animal activity.

We also examined whether a cat seen on a previous day influences the rat seen on the subsequent day. We calculated a lag period for cats by subtracting 1 day from when each rat was captured (e.g., we calculated the number of rat triggers as our response in the fitted line plot and used the lag of cats as the predictor). We also calculated a lag period for rats to determine whether their presence on a subsequent day predicted the number of cats.

All videos were reviewed and manually scored by the same observer. We first noted that the presence of the colony entrance

FIGURE 2 | Coat patterns of five feral cats and subsequent ethogram used to score their behaviors at a semi-enclosed industrial building in New York City from Dec. 27, 2017—May 28, 2018. Cat behaviors scored (modified from Stanton et al., 2015) include walking (w): forward locomotion where animal moves slowly. Running (r): animal uses forward locomotion at a rapid gait. Stalking (s): slow forward motion in crouched position, with head low and eyes focused on potential prey. Predation (p): hunting or killing that leads to a direct kill. Sniffing (s): cat smells floor or object by inhaling air through the nose. Feeding (f): animal ingests food, usually from floor. Sitting (s): animal is immobile in upright position. Marking (m): while standing with tail raised vertically, cat releases jet of urine backwards onto a substrate of an object. Tail may quiver as urine is released.

(harborage) was to the east (**Figure 1**) across the south wall, and the bolt exit (relief entrance when the main entrance is blocked) was to the North. Whereas, the typical foraging and water sources the animals utilized were refuse heaps to the west. While scoring cat videos, we recorded all instances of eight primary cat behaviors (**Figure 2**): walking, running, stalking, chasing (predation), sniffing, feeding, sitting, marking (or overmarking) and direction of movement of animal. As these videos varied in length, some for only seconds, we only recorded instances of an event, and not the duration of a behavior. While scoring rat videos (**Figure 3**), we recorded all instances of walking, running, freezing, drinking, feeding, sitting, and being chased/predation. Likewise, we recorded direction of movement of the animal. This work was performed under IACUC guidelines for ethical conduct in the care and use of nonhuman animals in research (Fordham IACUC JMS 17-01).

Statistics

We performed binary logistic regressions to assess whether the proportion of rat triggers were related to the proportion of cat triggers in a given day, whether the proportion of previous day cat sights was related to the proportion of rat sightings on the current day, whether the proportion of rat sightings the previous day was related to the proportion of cat sightings on the current day, and whether the percent of people triggers was associated with the proportion of rats in a given day. We also used binary logistic regressions to determine the relationships between cats and the 8 common rat behaviors (except chase/predation which only occurred 3 times) and direction of movements. Statistical significance (alpha) was set at $P \leq 0.05$ and all descriptive and inferential analyses were conducted using Minitab V. 17 (State College, PA).

FIGURE 3 | Anaesthetization equipment and typical >330 g rat captured along with ethogram used to score rat behaviors at a semi-enclosed industrial building in New York City from Dec. 27, 2017—May 28, 2018. Rat behaviors scored include walk (w): forward locomotion where animal moves slowly. Run(r): forward locomotion where animal moves at a faster pace, forearms extended. Freeze/stop (f): rats cease activity and become immobile, usually in response to a perceived threat. Drink (d): animal consumes water or other liquid source, usually from puddles. Sniff (s): animal smells object by inhaling air through the nose. Feed (f): animal ingests food particle, often from the floor surface. Sit (s): animal is in relaxed posture, immobile, and may be on haunches with forearms extended.

RESULTS

A total of 306 videos taken over 79 days were scored. Five individual cats were identified based on color, markings and scar patterns (**Figure 2**). These animals first appeared on December 27 and remained throughout the study. On five occasions, three of the cats appeared on the same day. There were three predation attempts by two cats (one each had a successful attempt) and one unsuccessful attempt. All five cats participated in stalking events (20).

During the period when cats were present (Dec 27–May 28), the number of cats on a given day influenced the likelihood of rats to be seen on the same day (**Figure 4**, $OR = 0.01$; 95% CI [0.00, 0.02], $p < 0.001$). For every 1% increase in the number of cats on a given day, it is 100 times less likely that a rat will trigger a camera on that day. Additionally, the number of cats on a previous day inversely predicted the number of rats ($OR = 0.15$; 95% CI [0.06, 0.34], $p < 0.001$). Every 1% increase in the number of cats seen on the previous day results in a rat being 6.6 times less likely to be seen on that day. The number of rats seen on a given day did not predict the number of cats seen on the following day (OR = 0.62, $p > 0.1$), nor did the number of people seen on a given day influence the likelihood of rats being seen on the same day (OR = 0.44, $p > 0.1$).

Behaviors and Space-Use

Two hundred fifty-nine instances of cat behaviors were recorded (**Table 1**). Among these behaviors, 46.3% (120) included walking in an eastward or westward direction beginning or ending near the rat colony entrance. Two videos culminated with the cat's head encroaching into the rat colony entrance. We found 27.4% of recorded cat behaviors (71) included sniffing. This behavior was performed across the open space floor and included two objects that were commonly over-marked. Two cats (**Figure 1**) overmarked the central object in the picture (the RFID enclosure) and occasionally perched on the unit, while the other three cats marked the bucket beside the colony entrance. There were 20 stalking attempts and 3 predation attempts, though cats and rats only appeared in the same video fewer than 10 times. Two predation attempts were successful when cats plucked the animal from under a hiding spot (beside the marking object), the lone predation attempt on the open floor was a failure when the rat stopped running, and the cat also ceased the chase, only to stare at it. Because the number of rats seen on a given day did not predict the number of cats seen on the following day, we did not assess number of rat sightings as predictors of any specific cat behavior.

On the other hand, because the number of cats did predict rat sightings, we examined how cat sightings varied with individual rat behaviors. The number of cats on a given day influenced the number of rats moving in an eastward direction toward their colony (**Table 2**; $OR = 1.19$; 95% CI [1.00, 1.41], $p < 0.05$). Walking, a more conservative measure of locomotion than potentially running into a predator, was the only other behavior to vary (positively) with cat sightings.

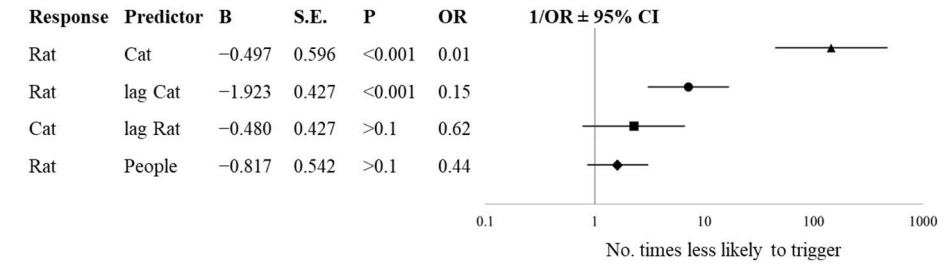

FIGURE 4 | Forest plot and likelihood estimates from binary logistic regressions with varied predictors at a semi-enclosed industrial building in New York City from Dec. 27, 2017—May 28, 2018. Lag refers to sightings on the preceding day.

TABLE 1 | Instances of recorded behavior of cats at a semi-enclosed industrial building in New York City from Dec. 27, 2017—May 28, 2018.

Cat ID	Black	Gray	Scars	Black/ white	White	Unknown	Total
BEHAVIOR							
Walking	14	46	6	21	27	6	120
Running	2	1	0	3	2	0	8
Stalking	1	3	2	9	5	0	20
Predation	0	1	0	1	1	0	3
Sniffing	8	45	2	6	10	0	71
Feeding	0	6	0	1	1	0	8
Sitting	3	3	0	0	2	0	8
Marking	7	3	2	4	5	0	21
DIRECTION							
North	1	3	1	5	14	2	26
South	2	2	0	1	1	1	7
East	8	9	3	14	14	1	49
West	3	36	4	5	4	3	54

Direction refers to direction focal animal moved out of view.

TABLE 2 | Likelihood estimates in Binary Logistic Regressions with number of cat sightings as predictor of behavior for rats at a semi-enclosed industrial building in New York City from Dec. 27, 2017—May 28, 2018.

Behavior	Coef.	SE	Z	P	OR	Lower	Upper
Walk	0.191	0.088	2.17	**0.030**	1.21	1.02	1.44
Run	−0.060	0.086	−0.70	0.483	0.94	0.80	1.11
Freeze	−0.144	0.159	−0.90	0.366	0.87	0.63	1.18
Drink	−17.46	5,568.95	−0.00	0.997	0.00	0.00	*
Sniff	0.0302	0.095	0.32	0.751	1.03	0.86	1.24
Feed	−17.521	3,990.70	−0.00	0.996	0.00	0.00	*
Sit	−17.466	5,568.95	−0.00	0.997	0.00	0.00	*
North	0.122	0.107	1.14	0.254	1.13	0.92	1.39
South	0.160	0.119	1.34	0.181	1.17	0.93	1.48
East	0.171	0.086	1.98	**0.047**	1.19	1.00	1.41
West	0.176	0.093	1.90	0.058	1.19	0.99	1.43

*Direction refers to orientation that rat moves when exiting video. Bold number indicates the number of cats influenced this behavior at $p < 0.05$. *Refers to upper limit bring too large to display i.e., > 1,000.*

DISCUSSION

This study is the first research to document the direct and indirect influences of identifiable feral cats on a partially-identified population of city rats. This partly-enclosed urban ecosystem was under heavy pressure from cats, as evidenced by the number of cats present and the number of staking attempts from each cat throughout the study. These events occurred during all hours of the day and evening. Yet of the 259 instances of behavior recorded by cats over 79 days/nights, only 3 times did a cat pursue a rat—and only once across an open floor. We were surprised that an area where up to three cats patrolled within the same 24-h period, would have so few direct predation attempts. While field cameras often have a latency period and are not perfect at capturing all behaviors, the cameras did have a fast 0.4 s trigger-speed and the back and forth nature of the chase lends a high likelihood of capturing at least part of the process on video.

We were, at first, surprised that such low predation could result in significant changes in space use and activity time. The odds ratios were very high at predicting rat sightings when the cat was sighted either during a given day ($OR = 0.01$), or a preceding day ($OR = 0.15$). We might have expected the rats to be habituated to cats in much the same way they seem to be to human visitation. This, despite the fact, that humans were regularly trapping and releasing rats and when extermination was ongoing as mandated by the city. However, it is also well known that all animals, especially potential prey, overestimate their risks using "simple rules of thumb" (Bouskila and Blumstein, 1992). It is better to falsely assume an organism is dangerous (a false positive) than to assume a dangerous animal is harmless (a false negative) (Johnson et al., 2013). This is primarily because not being sensitive to predation can lead to the ultimate loss of fitness via predation (Bouskila and Blumstein, 1992).

The two successful predation events were likely with smaller individuals under the weight range (<250 g) identified by Childs (1986). Unfortunately, these two individuals were not microchipped and thus we had no measure of weight for them. Additionally, these animals were both captured in hiding beside or beneath cats' preferred marking locations. Thus, it is also possible the rats were sick from disease or baiting from bromadiolone poison. While the researchers have longitudinal access to the study sight, the site owners are legislatively-obligated to continue baiting, even while supporting scientific research.

Baiting is a normal part of urban ecosystems in cities throughout the world, and this must be figured into any ecological findings.

At first it seemed counter-intuitive that walking would vary significantly and positively with the number of cats sighted. However, **Figure 1** shows that rats could either walk across their runway using cover as they moved east, or potentially run into an ambush. In this context, walking is the more conservative means of locomotion. The animals were more likely to move eastward during heavy cat sightings because this is where the primary shelter and colony entrance is located (**Figure 1**).

We were mildly disappointed that the RFID station and histories of the previously chipped rats were not more useful to our predation study. However, the RFID set-up still served to give some indication of the number of animals that were present in an active colony and to show that their weights were collectively much greater than the 250 g upper limit (Childs, 1986). Had the antennas been baited with an attractant, the rats might have been more likely to activate their identification tags prior to inducing/performing a specific behavior. As it happened, we were in the process of evaluating a putative scent-deterrent (Parsons et al., unpublished data).

Given our results, we can only note that the public's continued confusion between rats and mice (*Mus musculus and Peromyscus spp*.) may be encouraging a poor, but risky (Woinarski et al., 2017) approach to rat control. It is clear that although the rats were seen less frequently where we had our cameras placed (open areas), rats were still persisting in a densely-populated colony. Their space use patterns indicate they simply moved elsewhere for food that did not involve cat stalking attempts.

While our results are clear, they should be replicated in areas with a less than continuous food supply. Given the high reproductive rate of city rats(Davis, 1951), when sufficient food is available, one pregnant female can give birth to 6-10 litters of 12 pups each per year. In areas where less food is available, and thus rats are reproducing less frequently, it is possible that the direct effects of predation by cats could be more significant. Further experimental cat control (e.g., data with and without cat presence) is needed to test this phenomenon and to further characterize the effects of cats on rat mortality.

Given that small prey over-estimate risks from predators, further experimentation is also required as to how the presence of cats, or their scents, indirectly influences the feeding rate (Wernecke et al., 2016) and fecundity (Voznessenskaya, 2014) of rats, and whether this has any tangible benefit as an adjunct to rat control (Himsworth et al., 2013). Such information is essential if we are to understand whether the influence of feral cats on rats is remotely worth the risks to native urban wildlife. Our results at a waste recycling facility, however, suggest that city rats can persist in high density, simply by altering their movements, despite the presence of hunting cats.

DATA AVAILABILITY

The authors hope our study will lead to future efforts to quantify the impact of cats on rats. Thus, our data will be made available free of charge on request.

AUTHOR CONTRIBUTIONS

MP designed the study and wrote the article. MP and MD collected all data. PB and JM-S assisted the writing and edits.

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REFERENCES

Apfelbach, R., Parsons, M. H., Soini, H. A., and Novotny, M. V. (2015). Are single odorous components of a predator sufficient to elicit defensive behaviors in prey species? *Front. Neurosci.* 9:263. doi: 10.3389/fnins.2015.00263

Bouskila, A., and Blumstein, D. T. (1992). Rules of thumb for predation hazard assessment: predictions from a dynamic model. *Am. Nat.* 139, 161–176. doi: 10.1086/285318

Carthey, A. J., and Banks, P. B. (2018). Naïve, bold, or just hungry? An invasive exotic prey species recognises but does not respond to its predators. *Biol. Invasions* 1–13. doi: 10.1007/s10530-018-1782-4

Childs, J. E. (1986). Size-dependent predation on rats (*Rattus norvegicus*) by house cats (*Felis catus*) in an urban setting. *J. Mammal.* 67, 196–199. doi: 10.2307/1381025

Combs, M., Puckett, E. E., Richardson, J., Mims, D., and Munshi-South, J. (2018). Spatial population genomics of the brown rat *(Rattus norvegicus*) in New York City. *Mol. Ecol.* 27, 83–98. doi: 10.1111/mec.14437

Davies, H. F., McCarthy, M. A., Firth, R. S., Woinarski, J. C., Gillespie, G. R., Andersen, A. N., et al. (2017). Top-down control of species distributions: feral cats driving the regional extinction of a threatened rodent in northern Australia. *Divers. Distrib.* 23, 272–283. doi: 10.1111/ddi.12522

Davis, D. E. (1951). A comparison of reproductive potential of two rat populations. *Ecology* 32, 469–475. doi: 10.2307/1931724

Embar, K., Raveh, A., Burns, D., and Kotler, B. P. (2014). To dare or not to dare? Risk management by owls in a predator–prey foraging game. *Oecologia* 175, 825–834. doi: 10.1007/s00442-014-2956-0

Fitzgerald, B., Karl, B., and Veitch, C. (1991). The diet of feral cats (*Felis catus*) on Raoul Island, Kermadec Group. *New Zealand J. Ecol.* 15, 123–129.

Frank, A. S., Johnson, C. N., Potts, J. M., Fisher, A., Lawes, M. J., Woinarski, J. C., et al. (2014). Experimental evidence that feral cats cause local extirpation of small mammals in Australia's tropical savannas. *J. Appl. Ecol.* 51, 1486–1493. doi: 10.1111/1365-2664.12323

Glanton, D. (2017). *Chicago's Rat Problem Gnaws at Feline Fears, Chicago Tribune.* Chciago, IL.

Griffith, D. A., and Wong, D. W. (2007). Modeling population density across major US cities: a polycentric spatial regression approach. *J. Geogr. Syst.* 9, 53–75. doi: 10.1007/s10109-006-0032-y

Herman, C. S., and Valone, T. J. (2000). The effect of mammalian predator scent on the foraging behavior of *Dipodomys merriami*. *Oikos* 91, 139–145. doi: 10.1034/j.1600-0706.2000.910113.x

Himsworth, C. G., Feng, A. Y., Parsons, K., Kerr, T., and Patrick, D. M. (2013). Using experiential knowledge to understand urban rat ecology: a survey of Canadian pest control professionals. *Urban Ecosyst.* 16, 341–350. doi: 10.1007/s11252-012-0261-4

Hurst, J. L., and Beynon, R. J. (2004). Scent wars: the chemobiology of competitive signalling in mice. *Bioessays* 26, 1288–1298. doi: 10.1002/bies.20147

Jeschke, J. M., Kopp, M., and Tollrian, R. (2002). Predator functional responses: discriminating between handling and digesting prey. *Ecol. Monogr.* 72, 95–112. doi: 10.1890/0012-9615(2002)072[0095:PFRDBH]2.0.CO;2

Johnson, D. D., Blumstein, D. T., Fowler, J. H., and Haselton, M. G. (2013). The evolution of error: Error management, cognitive constraints, and adaptive decision-making biases. *Trends Ecol. Evol.* 28, 474–481. doi: 10.1016/j.tree.2013.05.014

Karl, B. J., and Best, H. A. (1982). Feral cats on Stewart Island; their foods, and their effects on kakapo. *New Zealand J. Zool.* 9, 287–293. doi: 10.1080/03014223.1982.10423857

Kauhala, K., Talvitie, K., and Vuorisalo, T. (2015). Free-ranging house cats in urban and rural areas in the north: useful rodent killers or harmful bird predators? *Folia Zool.* 64, 45–55. doi: 10.25225/fozo.v64.i1.a6.2015

Kelliher, K. R., and Wersinger, S. R. (2009). Olfactory regulation of the sexual behavior and reproductive physiology of the laboratory mouse: effects and neural mechanisms. *ILAR J.* 50, 28–42. doi: 10.1093/ilar.50.1.28

Kikillus, K. H., Chambers, G. K., Farnworth, M. J., and Hare, K. M. (2017). Research challenges and conservation implications for urban cat management in New Zealand. *Pac. Conserv. Biol.* 23, 15–24. doi: 10.1071/PC16022

Loss, S. R., and Marra, P. P. (2017). Population impacts of free-ranging domestic cats on mainland vertebrates. *Front. Ecol. Environ.* 15:1633. doi: 10.1002/fee.1633

Loss, S. R., Will, T., and Marra, P. P. (2013). The impact of free-ranging domestic cats on wildlife of the United States. *Nat. Commun.* 4:1396. doi: 10.1038/ncomms2380

Montague, M. J., Li, G., Gandolfi, B., Khan, R., Aken, B. L., Searle, S. M., et al. (2014). Comparative analysis of the domestic cat genome reveals genetic signatures underlying feline biology and domestication. *Proc. Natl. Acad. Sci. U.S.A.* 111, 17230–17235. doi: 10.1073/pnas.1410083111

Naidenko, S. V., Naidenko, S. V., Clark, L., and Voznessenskaya, V. V. (2003). *Predator Presence Affects the Reproductive Success of Prey in Outdoor Conditions.* Lincoln, NE: USDA

Orrock, J. L., and Danielson, B. J. (2009). Temperature and cloud cover, but not predator urine, affect winter foraging of mice. *Ethology* 115, 641–648. doi: 10.1111/j.1439-0310.2009.01654.x,

Orrock, J. L., Danielson, B. J., and Brinkerhoff, R. J. (2004). Rodent foraging is affected by indirect, but not by direct, cues of predation risk. *Behav. Ecol.* 15, 433–437. doi: 10.1093/beheco/arh031

Parsons, M. H., Banks, P. B., Deutsch, M. A., Corrigan, R. F., and Munshi-South, J. (2017). Trends in urban rat ecology: a framework to define the prevailing knowledge gaps and incentives for academia, pest management professionals (PMPs) and public health agencies to participate. *J. Urban Ecology* 3:jux005. doi: 10.1093/jue/jux005

Parsons, M. H., and Blumstein, D. T. (2010). Feeling vulnerable? Indirect risk cues differently influence how two marsupials respond to novel dingo urine. *Ethology* 116, 972–980. doi: 10.1111/j.1439-0310.2010.01810.x

Parsons, M. H., Sarno, R., and Deutsch, M. (2015). Jump-starting urban rat research: conspecific pheromones recruit wild rats into a behavioral and pathogen-monitoring assay. *Front. Ecol. Evol.* 3:146. doi: 10.3389/fevo.2015.00146

Parsons, M. H., Sarno, R. J., and Deutsch, M. A. (2016). A detailed protocol to enable safe-handling, preemptive detection, and systematic surveillance of rat-vectored pathogens in the urban environment. *Front. Public Health* 4:132. doi: 10.3389/fpubh.2016.00132

Pillay, K. R., Streicher, J., and Downs, C. T. (2018). Home range and habitat use of feral cats in an urban mosaic in Pietermaritzburg, KwaZulu-Natal, South Africa. *Urban Ecosyst.* 1–11. doi: 10.1007/s11252-018-0766-6

Puckett, E. E., Park, J., Combs, M., Blum, M. J., Bryant, J. E., Caccone, A., et al. (2016). Global population divergence and admixture of the brown rat (*Rattus norvegicus*). *Proc. R. Soc. B* 283:20161762. doi: 10.1098/rspb.2016.1762

Rozzi, R., and Lomolino, M. V. (2017). Rapid dwarfing of an insular mammal–the feral cattle of amsterdam island. *Sci. Rep.* 7:8820. doi: 10.1038/s41598-017-08820-2

Schmidt-Nielsen, B., and O'Dell, R. (1961). Structure and concentrating mechanism in the mammalian kidney. *Am. J. Physiol.* 200, 1119–1124. doi: 10.1152/ajplegacy.1961.200.6.1119

Siegel, P. S., and Stuckey, H. L. (1947). The diurnal course of water and food intake in the normal mature rat. *J. Comparative Physiol. Psychol.* 40:365. doi: 10.1037/h0062185

Stanton, L. A., Sullivan, M. S., and Fazio, J. M. (2015). A standardized ethogram for the felidae: a tool for behavioral researchers. *Appl. Anim. Behav. Sci.* 173, 3–16. doi: 10.1016/j.applanim.2015.04.001

Takács, S., Gries, R., Zhai, H., and Gries, G. (2016). The sex attractant pheromone of male brown rats: identification and field experiment. *Angew. Chem. Int. Edn.* 55, 6062–6066. doi: 10.1002/anie.201511864

Themb'alilahlwa, A., Monadjem, A., McCleery, R., and Belmain, S. R. (2017). Domestic cats and dogs create a landscape of fear for pest rodents around rural homesteads. *PLoS ONE* 12:e0171593. doi: 10.1371/journal.pone.0171593

Voznessenskaya, V. V. (2014). Influence of cat odor on reproductive behavior and physiology in the house mouse (*Mus musculus*). *Neurobiol. Chem. Commun.* 389–406. doi: 10.1201/b16511-15

Voznessenskaya, V. V., Naidenko, S. V., Feoktistova, N. Y., Krivomazov, G. J., Miller, L. A., and Clark, L. (2003). *Predator Odours as Reproductive Inhibitors for Norway Rats. USDA National Wildlife Research Center-Staff Publications*. Boca Raton, FL: CRC Press.

Wernecke, K. E., Brüggemann, J., and Fendt, M. (2016). Predator odor exposure increases food-carrying behavior in rats. *Physiol. Behav.* 154, 15–19. doi: 10.1016/j.physbeh.2015.11.005

Woinarski, J., Murphy, B., Legge, S., Garnett, S., Lawes, M., Comer, S., et al. (2017). How many birds are killed by cats in Australia? *Biol. Conserv.* 214, 76–87. doi: 10.1016/j.biocon.2017.08.006

Yip, S. J., Rich, M.-A., and Dickman, C. R. (2015). Diet of the feral cat, *Felis catus*, in central Australian grassland habitats during population cycles of its principal prey. *Mammal Res.* 60, 39–50. doi: 10.1007/s13364-014-0208-7

Zhou, N., Fridley, D., Khanna, N. Z., Ke, J., McNeil, M., and Levine, M. (2013). China's energy and emissions outlook to 2050: Perspectives from bottom-up energy end-use model. *Energy Policy* 53, 51–62. doi: 10.1016/j.enpol.2012.09.065

Zielinski, W. J., vom Saal, F. S., and Vandenbergh, J. G. (1992). The effect of intrauterine position on the survival, reproduction and home range size of female house mice (*Mus musculus*). *Behav. Ecol. Sociobiol.* 30, 185–191. doi: 10.1007/BF00166702

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Public Complaints Reflect Rat Relative Abundance Across Diverse Urban Neighborhoods

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Preventing infestations of rats is crucial for minimizing property damage and the transmission of rat-associated pathogens to humans. Due to the logistical challenges in assessing rat abundance over large areas, public officials must often use the number of public rat complaints to estimate the relative abundance of rats and the subsequent need for rodent control. However, the likelihood of reporting complaints may be driven by socioeconomic factors and therefore may not accurately reflect rat abundance. In this study, we tested whether the number of rat complaints reflect rat relative abundance and if rat complaints and abundance are higher in alleys with greater levels of harborage, food attractants, and poor structural integrity. We conducted this study in Chicago, IL, USA where public rat complaints have risen by 39% from 2008 up to 45,887 in 2017, and where socioeconomic factors vary considerably across neighborhoods. We assessed municipal rat complaints, census data, and land cover data for 77 community areas across Chicago. In collaboration with pest management professionals, we trapped brown rats (*Rattus norvegicus*) in alleys in 13 community areas that varied from low to high measures of household income and urban development. At trapping sites, we recorded signs of rat activity, attractants, and infrastructure condition. Based on candidate model comparisons using linear models, we found that rat complaints were most associated with rat trap success. Rat trap success was most associated with increasing complaints, percent of rented housing units, and decreasing vacant land. At a local scale, alleys with more complaints and higher trap success also had more uncontained garbage. Our results demonstrate that, at least in Chicago, public reporting can serve as a useful tool to identify areas of greater rat activity for targeted control efforts. Our study also suggests the need for habitat modification to minimize access to attractants. Finally, our results highlight how partnerships between researchers and private practitioners can facilitate large-scale projects on rat infestation risks in urban areas.

Keywords: brown rat, urban wildlife, rodent abundance, rodent control, public complaints, alley

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INTRODUCTION

Brown rats (*Rattus norvegicus*) are one of the most abundant and broadly-distributed wildlife species in urban areas worldwide, and frequently come into conflict with residents due to property damage and the spread of zoonotic diseases. Rats have been estimated to cause over $27 billion USD in damage to consumer goods each year in the US (Pimentel, 2007) and some rat-associated zoonoses, such as leptospirosis, are increasing globally (Panti-May et al., 2016; Richardson et al., 2017). To mitigate these risks and costs, municipal governments invest heavily in public education and rodent control; nearly one billion dollars is spent annually on rodenticide products in the United States (Specialty Consultants, 2016).

To effectively mitigate rat-associated risks in urban environments, recent work has examined the environmental factors associated with urban rat abundance. Identifying features that promote infestations across spatial scales is important as higher rat densities are presumed to increase the risk of disease transmission and property damage (Rael et al., 2016). In cities, rat populations or signs of rat activity are often higher in areas with lower incomes and higher rates of building abandonment or vacancy (Himsworth et al., 2014; Johnson et al., 2016; Rael et al., 2016). Thus, the abundance of rats and subsequent risk of property damage and disease transmission is likely heterogeneous across urban neighborhoods. However, most previous studies have examined rats within areas presumed to be high risk (e.g., Himsworth et al., 2013; Panti-May et al., 2016; Rael et al., 2016, but see Ayral et al., 2015), therefore, little is known about changes in rat abundance and associated risks over large spatial scales across diverse urban neighborhoods.

Because it is challenging and expensive to systematically survey large urban areas for signs of rats (Desvars-Larrive et al., 2018), municipal governments must often estimate rat abundance and the need for rodent control based on public rat complaints. However, this assumed relationship between complaints and rat abundance may instead reflect many other factors and may not accurately reflect rat abundance. For example, there may be an intuitive relationship between the abundance of people and the number of complaints. People may also be more likely to report rats based on their own knowledge and attitudes, which has been shown to occur with reporting wildlife conflict from other species (e.g., black bears *Ursus americanus*; Howe et al., 2010). Specifically, residents may be more likely to complain if they are able to identify signs of rat infestation, are concerned about the risks posed by rat infestations, are aware of how to report complaints, and believe the report will result in action. Similarly, rental tenants might be less likely to report rats, or may instead make complaints to their building managers due to fears of landlord reprisal (Bachelder et al., 2016). The seasonal distribution of rat complaints may also follow seasonal patterns of rat abundance (i.e., peak in late summer in temperate regions (Feng and Himsworth, 2014) or when favorable weather increases human outdoor activity, leading to more rat observations (Hume et al., 2002). Previous analyses of rat sightings suggest that, similarly to observed trends in rat abundance, observations of rats tend to increase with vacant housing, building age, and in areas with lower education levels (Walsh, 2014) and near food attractants (Ayyad et al., 2018). However, it is important to validate any relationship between rat sightings and rat abundance to improve the efficacy and efficiency of rat mitigation efforts.

We studied rats and complaints made about them in the city of Chicago, Illinois, USA, where there appears to be increasing public concern about rat infestations. Chicago reportedly has the most rat complaints per capita of any US city, according to recent estimates of 311 calls (Renthop, 2018). In response to these concerns, the municipal government increased rat control efforts by over $1 million USD in 2018 (Cherone, 2017) and used public complaints to identify areas in need of rodent control (i.e., rodenticide baiting and trapping). This increase in rat control enabled us to compare rates of rat complaints and trap success across Chicago's neighborhoods. In addition to increasing public complaints and rat control efforts, Chicago is an ideal system to explore variation in complaints and rat abundance because Chicago's neighborhoods vary markedly by several socioeconomic and environmental metrics (Perkins and Sampson, 2015). For example, median annual household incomes range from $<$ $15,000 to over $100,000 and resident population density ranges from 59 to 1,241 people/km^2 (CMAP, 2017). Thus, the rates of both rat infestations and complaints about rats may vary significantly.

In this study, we tested the assumption that rat complaints reflect the relative abundance of rats across urban neighborhoods. We did so by comparing rates of rat complaints with measures of trap success acquired through a partnership with a private pest control company in 13 community areas across Chicago along gradients of income and urban development. Using the results of this analysis, we then predicted relative rat abundance across the city from complaints. We then investigated correlates of rat abundance and complaints at two spatial scales by collecting socioeconomic data from community areas and recording rat attractants and habitat characteristics in alleys. Lastly, we tested whether the annual distribution of rat complaints was consistent with seasonal changes in rat abundance based on natural history. Our results can be used to predict areas and times of high relative rat abundance to prioritize rat control and mitigate property damage and the spread of rat-associated diseases.

MATERIALS AND METHODS

Study Area and Rat Complaint Dataset

Chicago is a large city of 2.7 million people (United States Census Bureau, 2016) that borders Lake Michigan and has a humid continental climate (average summer high = 24.3°C; average winter high = −0.3°C; National Oceanic and Atmospheric Administration, 2015). Chicago is composed of over 200 neighborhoods that have developed over time but has been officially divided into 77 community areas, which are static and often contain multiple neighborhoods. We studied rat complaints first at the scale of community areas, rather than neighborhoods, because community areas do not change over

time and are recognized by the city of Chicago for census and urban planning purposes (City of Chicago, 2010). We also chose this spatial scale because rat complaints varied more between community areas than between neighborhoods within community areas [F-test of equality of variances: $F_{(76,76)} = 15.7$, $p < 0.001$].

The 77 community areas of Chicago are highly variable in terms of socioeconomics and demographics. We accessed socioeconomic and land cover data for all community areas through the Chicago Metropolitan Agency for Planning (CMAP) database of Community Data Snapshots. This dataset summarizes demographic and land use data from the U.S. Census Bureau's 2011–2015 American Community Survey and CMAP's 2013 Land Use Inventory. We then extracted data that have been previously associated with rat activity (e.g., Childs et al., 1998; Ayral et al., 2015) to test whether socioeconomic factors such as household income, rented vs. owned properties, proportion of vacant city-owned land (e.g., vacant lots), and population density, would influence rates of complaints (**Table 1**).

We accessed all rat complaints made to the City of Chicago Bureau of Rodent Control from January 1, 2008 to April 30, 2018. All complaint records contained the date and UTM coordinates to facilitate seasonal and spatial analyses. The complaints did not contain identifying information such as name or address. When comparing the distribution of complaints with trap success we only included the last 12 months of complaints as these distributions may change across years.

Rat Trapping

Rats were trapped as part of rat control operations by Landmark Pest Management (hereafter Landmark). Trapping effort for this study took place in pairs of alleys in 13 community areas throughout Chicago from April 20 to June 20, 2018 (**Figure 1A**). These community areas were chosen to represent gradients across socioeconomic variables and land use while attempting to minimize confounding relationships between these variables (**Figure 1**, **Table 1**).

In each community area, rats were trapped in the two alleys with the highest levels of complaints, indicated by the city of Chicago Department of Streets and Sanitation. We targeted these alleys to maximize trap success for a concurrent study on rat pathogens and stress across community areas. Although we targeted areas with high numbers of complaints within each community area, there was considerable variation in complaints within the past 12 months within 1 km of trapping alleys in different community areas (range = 7–128).

At each designated trapping alley, Landmark set 10 enclosed snap trap stations (pairs of Victor snap traps in JT Eaton aluminum stations) and baited them with canned cat food following standard procedures. Within an alley, trap stations were on average 15 m apart (ranged between 13 and 18) and checked at least every 48 h. Any trapped rats were double bagged, labeled with the date and alley location ID, and frozen at −20°C. We recorded the start and end dates of the trapping period and any trap losses due to theft to calculate trap success as rats trapped per trap night and per trap set (mean ± S.D. number of trap nights = 54 ± 3, range = 47–57). While accounting for sprung traps (those that are set off for any reason (e.g., rats, non-target species, false closure) is preferred to calculate trap success (Norton, 1987), it was unfortunately not feasible in our study but was uncommon. Therefore, we used the number of rats caught per trap night as an indicator of relative rat abundance (Panti-May et al., 2016).

Habitat Assessments

To test which local habitat features were most associated with higher rat complaints, we visited trapping alleys within 2 weeks of trapping to record rat attractants, harborage, and property structural integrity (**Figure 2**). We recorded the availability of

TABLE 1 | Characteristics of 13 community areas in Chicago, IL, USA used to model public rat complaints as a function of rat trap success and several socioeconomic and landscape variables.

Community area name	Human population Density (residents/km^2)	Median household income (USD)	Percent units rented (%)	Percent vacant land (%)	Complaints since 2008	Complaints 2017–2018	Pooled trap success (Rats/trap/night)
Armor Square	507	24,336	64.4	2.3	1,062	119	0.063
Beverly	234	90,766	16.9	0.5	964	154	0.018
Englewood	131	86,300	20.9	0.1	454	34	0.000
Forest Glen	207	101,559	10.6	0.3	1,979	404	0.001
Hegewisch	62	50,338	29.1	12.7	237	42	0.000
Lake View	1,126	76,854	63.8	0.5	17,402	1,845	0.121
Logan Square	737	59,216	63.4	1.6	19,088	1,944	0.094
Near North Side	292	78,290	51.8	7.3	4,818	600	0.073
North Lawndale	206	51,818	49.4	0.5	6,936	953	0.055
Roseland	209	28,504	61.1	28.5	4,628	575	0.021
South Lawndale	402	26,425	77.0	4.4	6,021	1,056	0.045
Washington Park	285	22,085	84.2	17.3	934	128	0.045
West Ridge	734	46,091	53.4	0.5	14,362	1,642	0.108

garbage in three ways: the number of garbage receptacles, the proportion of receptacles with holes large enough to permit rats (i.e., 2 cm in diameter), and uncontained garbage on an ordinal scale (0 = none, 1 = scattered pieces, 2 = piles or bags of garbage). We also recorded the presence or absence of dog waste because it is an anecdotal rat attractant. We quantified the abundance of clutter that could serve as rodent harborage (e.g., pallets, old furniture, vegetation) on an ordinal scale (0 = none, 1 = scattered items, 2 = piles). Lastly, we recorded the condition of the buildings (i.e., structural integrity of foundation, walls, and doors) and the surrounding grounds (i.e., structural integrity of concrete and asphalt, management of vegetation, presence of garbage and harborage) on an ordinal scale from 1 (poor) to 5 (excellent). A score of 1 indicated cracks in foundations, buckled concrete forming holes, many gaps under doors, broken windows, holes in walls, and unmanaged vegetation while a 5 indicated no uncontained garbage or harborage, sealed doors, and garbage receptacles clean and secured. We also recorded visible signs of active rat infestations such a sebum rub marks, gnaw marks on garbage cans and doors, tracks, and feces.

Because our trapping alleys were chosen based on high levels of complaints (mean number of complaints in 2017–2018 within 1 km = 42 ± 36 S.D.), we also visited two other randomly chosen alleys in each community area to increase our sample and facilitate comparisons to areas with fewer complaints (random alleys: 32 ± 32 complaints within 1 km). These alleys were chosen to be between 1 and 2 km from the trapping alley to reduce spatial autocorrelation but maintain similar socioeconomic and landscape context.

Statistical Analyses

We used linear models to test whether complaints were most correlated with rat relative abundance, or if complaints were more associated with socioeconomics and other factors. To do so we treated the number of rat complaints in each community area within the past 12 months (April 30, 2017–April 30, 2018) as the

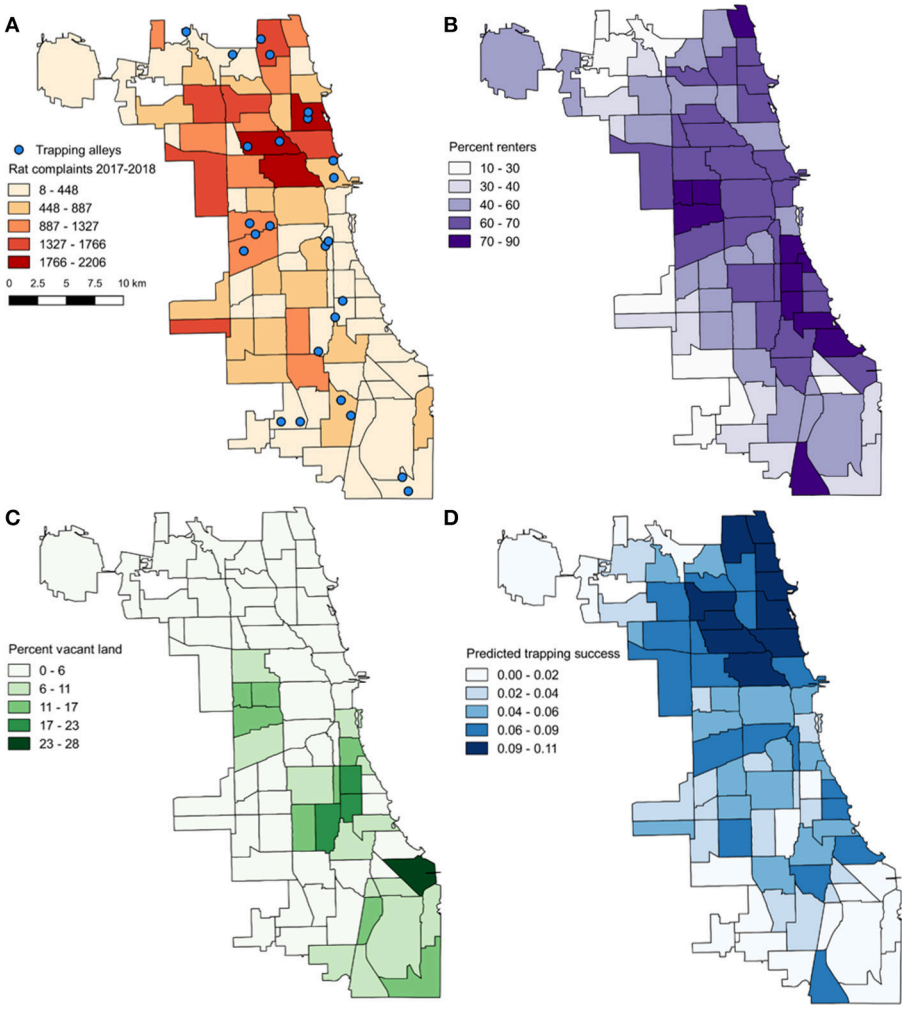

FIGURE 1 | Map of the city of Chicago (IL, USA) outlining the 77 community areas. Rats were trapped in pairs of alleys in 13 communities selected along gradients in rat complaints and several socioeconomic and landscape variables **(A)**. Rat trap success was most associated with complaints **(A)**, percent of rented housing units **(B)**, and percent vacant city-owned land **(C)**. Based on our model of these factors, we predict rat trap success across all community areas **(D)**.

response variable and generated a suite of six candidate models that represent different hypotheses about how rat complaints may vary across Chicago (**Table 2**). These six candidate models were composed of the following explanatory variables: rat trap success (rats captured per trap per night traps were active), human population density (individuals per km^2), and three indicators of socioeconomic status [median annual household income (USD), percent of rented residences (%), and percent city-owned vacant land (%)]. With these six candidate models, we tested whether the number of rat complaints varied as a function of: (1) rat abundance, (2) socioeconomics (income, percent renters, vacant land), (3) human population density, (4) rat abundance and human population density, (5) rat abundance, socioeconomics, and human population density (global model) and (6) the null model. We used Akaike's information criteria corrected for a small sample size (AICc) to compare the relative quality of each candidate model given the data. When estimating trap success rates, we pooled the number of rats captured across both alleys,

FIGURE 2 | We visited alleys with varying numbers of complaints in 13 community areas of Chicago to estimate any relationships between microhabitat and rat abundance (trap success) or the number of complaints within 1km. We characterized the abundance of uncontained garbage on an ordinal scale as none **(A)**, pieces **(B)**, or piles **(C)**. We also assessed the amount of harborage from vegetation **(E)** or clutter **(F)** and access to buildings through the structural integrity of concrete in and around foundations **(H)** and of walls **(I)**. Assessments of these three factors cumulated into an overall condition score from 1 to 5 with sites in poor condition **(C,F,I)** having high levels of uncontained garbage, harborage, and poor structural integrity while those in excellent condition had no visible issues **(A,D,G)**.

TABLE 2 | Comparison of candidate models used to predict rat complaints as a function of rat relative abundance (trap success), socioeconomics, and landscape variables across 13 community areas in Chicago, IL, USA.

Model	Covariates	df	AICc	Δ AICc	weight	Adjusted R^2
Rat relative abundance	Trap success	3	199.42	0.00	0.68	0.67
Population density	Human population density	3	202.12	2.70	0.18	0.65
Rats and population density	Trap success + human population density	4	202.55	3.13	0.14	0.67
Null	Intercept only	2	211.37	11.96	0.00	na
Socioeconomics	Income + vacancy rates + percent renters	5	216.34	16.92	0.00	0.30
Global	Trap success + human population density + income + vacancy rates + percent renters	7	225.64	26.22	0.00	0.53

which were highly correlated with the average trap success across the two alleys ($R^2 = 0.95$).

Based on the most important variables in this model comparison, we then compared a similar set of candidate models with trap success as the response variable. We then used the top-ranked model for trap success to predict rat abundance across all 77 community areas (**Table 2**). In one of these candidate models, complaints were highly correlated with human population density. To reduce multi-collinearity we fit a simple linear regression between these variables, treating complaints as the response and human population density as the independent variable. Following this, we calculated the residuals of this regression and used them in place of human population density, with positive values indicating areas with higher human population density than expected given the number of complaints.

We also compared a suite of eight candidate models to evaluate alley characteristics most associated with rat complaints and trap success. These candidate models had either the number of rat complaints within 1 km of the trapping alley or the trap success at that particular alley as response variables and six independent variables we measured in the field (see *Habitat assessments*; **Table 4**). With these candidate models we tested whether rat complaints and/or relative abundance increased with (1) uncontained garbage, (2) the number of accessible garbage receptacles, (3) the presence of dog waste, (4) harborage, (5) building condition, (6) grounds condition, (7) the global model, and (8) the null model. We ranked these models using AICc to determine the most important predictors of rat activity.

Lastly, we tested whether the annual distribution of rat complaints over the past 10 years peaked in late summer, consistent with the period when rat population density is highest. To do so, we used Rayleigh's test of uniformity of circular data using the Circstats package in R (Lund and Agostinelli, 2018). Independent variables were not centered or scaled for any of the aforementioned analyses.

RESULTS

Correlates of Rat Complaints Across Community Areas

We accessed 369,581 complaints made to the Bureau of Rodent Control in Chicago from January 1, 2008 to April 30, 2018. Of these, 12.4% (45,887) were made between April 30, 2017 and April 30, 2018. Yearly rat complaints increased by 39% between 2008 and 2017 (35,410 to 49,043 complaints).

We captured 61 brown rats in 13 community areas from April 20 to June 20, 2018. Rat trap success ranged from 0 to 0.19 rats per trap night across alleys (mean = 0.05 ± 0.06 S.D.). Based on our candidate model comparisons, the number of rat complaints in a community area was most associated with trap success (**Table 2**). A 0.1 increase in trap success (two rats in a 48 h period; our median value) resulted in a 1,210.8 ± 325.3 increase in complaints made ($p = 0.003$). The second and third-most top models had similar fit (ΔAICc ≤ 4.0) and contained trap success and residual population density.

Predictors of Rat Relative Abundance Across Community Areas

Because we found a high correlation between complaints and trap success, we then predicted trap success across all 77 community areas using the number of complaints from April 2017 to April 2018. To also evaluate the importance of environmental and

TABLE 3 | Comparison of candidate models to predict rat trap success as a function of public rat complaints and several socioeconomic and landscape variables across 13 community areas in Chicago, IL, USA.

Model	df	AICc	Δ AICc	weight	Adjusted R^2
Complaints + renters + vacancy	5	−56.37	0	0.89	0.84
Complaints + human population density	4	−50.26	6.11	0.04	0.65
Complaints + income + renters + vacancy	6	−49.91	6.46	0.04	0.84
Complaints	3	−49	7.37	0.02	0.52
Complaints + vacancy	4	−46.71	9.66	0.01	0.55
Complaints + renters	4	−46.23	10.14	0.01	0.53
Complaints + human population density + income + renters + vacancy (Global)	7	−39.64	16.73	0.00	0.82
Complaints + income	4	−44.68	11.69	0.00	0.47
Complaints + income + renters	5	−43.88	12.49	0.00	0.59
Complaints + income + vacancy	5	−42.98	13.39	0.00	0.56
Null	2	−41.89	14.48	0.00	na

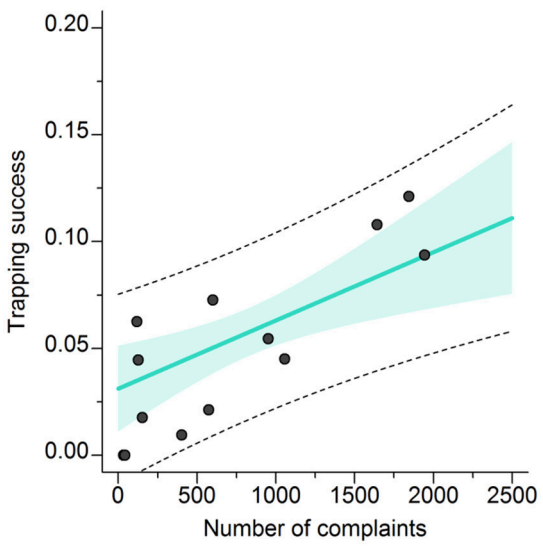

FIGURE 3 | Relationship between the number of rat complaints (between April 1 2017–April 1 2018) and trap success (rats captured per trap per night). The turquoise line shows the fitted line from our top model with the associated 95% confidence interval (shaded region) and 95% predicted interval (dashed lines).

socioeconomic factors, we compared a suite of 10 candidate models with trap success as the response variable and rat complaints and either human population density, income, vacant land, or percent of rented households as independent variables (**Table 3**). Trap success was most associated with the number of complaints, proportion of rented housing units, and proportion of vacant land (**Figures 1A–C**, **3**). Trap success increased with complaints ($\beta_{Complaints} = 1.86 \times 10^{-5} \pm 8.95 \times 10^{-6}$, $t = 2.1$, $p = 0.05$) and the proportion of renters ($\beta_{proportionrenters} = 0.13 \pm 0.003$, $t = 4.8$, $p = 0.0015$) but decreased with increasing vacant land ($\beta_{vacantland} = -0.41 \pm 0.009$, $t = -4.5$, $p = 0.0013$; Adjusted $R^2 = 0.84$, $F = 22.58$ (3.9), $p = 0.002$; **Table 3**). Using this model, we predicted trap success for all community areas across Chicago based on the number of complaints in 2017–2018, the proportion of rented units, and proportion of vacant land (**Figure 1D**). Trap success was predicted to be higher on the Northeastern side of Chicago relative to other parts of the city.

Correlates of Rat Complaints and Relative Abundance in Alleys

We visited 52 alleys within 2 weeks of the trapping period. Our top-ranked model was the global model (**Table 4**). Complaints near alleys increased with uncontained garbage ($\beta_{Garbage} = 23.1 \pm 8.3$, $t = 2.8$, $p = 0.008$), accessible garbage receptacles ($\beta_{Receptacles} = 1.0 \pm 0.4$, $t = -2.3$, $p = 0.03$), harborage ($\beta_{Harborage} = 1.1 \pm 4.5$, $t = 0.2$, $p = 0.81$), and building condition ($\beta_{Building} = 3.8 \pm 5.9$, $t = 0.6$, $p = 0.53$), but decreased with grounds condition ($\beta_{Grounds} = 4.5 \pm 7.8$, $t = 0.6$, $p = 0.56$) and dog waste ($\beta_{Dog} = -22.6 \pm 12.2$, $t = -1.9$, $p = 0.07$). Of the individual variables, uncontained garbage was the most important predictor of complaints ($t = 2.67$, $p = 0.01$; **Figure 4**). For trap success, the top-ranked model was uncontained garbage ($\beta_{Garbage} = 0.2 \pm 0.1$, $t = 1.7$, $p = 0.09$) however it was <4 AICc from all models apart from the global and null models (**Table 4**).

Seasonal Variation in Rat Complaints

The frequency of rat complaints peaked in August and the distribution of complaints was significantly non-uniform throughout the year (Rayleigh test for circular uniformity $Z = 20842.0$ $p < 0.0001$; concentration = 0.489, length of vector = 0.28 in August; **Figure 5**).

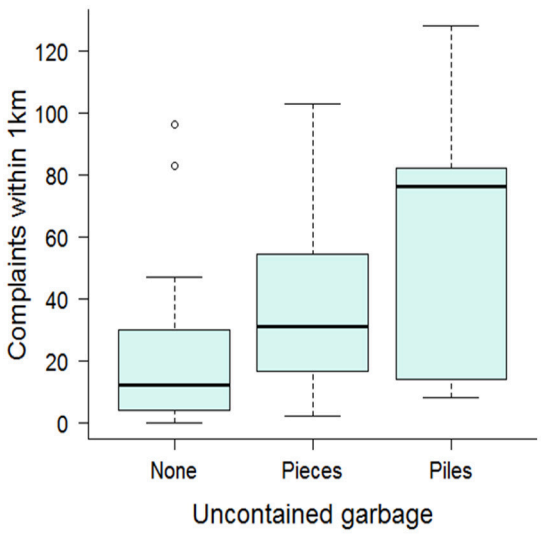

FIGURE 4 | Boxplot showing the median and quartile values of rat complaints in relation to uncontained garbage in alleys in Chicago, IL, USA. Uncontained garbage was recorded on an ordinal scale (0 = none, 1 = pieces, 2 = piles) and rat complaints were summed within 1 km of the alley.

TABLE 4 | Comparison of candidate models to predict rat complaints or trap success as a function of available attractants, harborage, structural integrity of buildings, and the condition of grounds in alleys.

Response variable	Model	df	AICc	Δ AICc	Likelihood	Weight	Multiple R^2	Adjusted R^2
Complaints within 1 km of alley	Global	8	424.0	0.0	1.0	0.4	0.31	0.2
	Uncontained garbage	3	424.1	0.1	1.0	0.4	0.13	0.11
	Null (Intercept)	2	428.0	4.0	0.1	0.1	na	na
	Accessible garbage receptacles	3	428.6	4.6	0.1	0.0	0.03	8.0×10^{-3}
	Harborage	3	429.0	5.0	0.1	0.0	2.3×10^{-3}	-7.0×10^{-4}
	Dog Waste presence	3	429.0	5.0	0.1	0.0	0.02	4.0×10^{-5}
	Building condition	3	429.6	5.6	0.1	0.0	9.0×10^{-3}	-1.5×10^{-3}
	Grounds condition	3	430.0	6.0	0.0	0.0	6.0×10^{-4}	-2.3×10^{-3}
Trapping success	Uncontained garbage	3	−60.8	0.0	1.0	0.3	0.13	0.08
	Dog Waste presence	3	−60.6	0.2	0.9	0.3	0.12	0.08
	Grounds condition	3	−59.2	1.6	0.4	0.2	0.06	0.01
	Building condition	3	−58.5	2.3	0.3	0.1	0.03	−0.02
	Harborage	3	−58	2.8	0.2	0.1	9.0×10^{-3}	−0.04
	Accessible garbage receptacles	3	−57.8	3.0	0.2	0.1	1.2×10^{-4}	−0.05
	Null	2	−56.8	4.0	0.1	0.0	na	na
	Global	8	−56.2	4.6	0.1	0.0	0.32	0.05

We visited rat trapping alleys and two randomly chosen alleys within 2 weeks of trapping in each of the 13 target community areas in Chicago, IL, USA.

DISCUSSION

Controlling rat populations is a priority for public health and safety in cities around the world. In this study, we investigated whether public rat complaints can serve as a reliable indicator of rat abundance to predict where rodent control is most needed. By comparing municipal rat complaints with rat trap success over 13 diverse community areas in Chicago, we found that complaints were highly correlated with relative abundance of rats. Trap success was greater in community areas with more complaints, a higher proportion of rented units, a lower proportion of vacant property, and in alleys with more uncontained garbage. We also found that complaints varied seasonally, peaking in late summer.

Although there are a number of socioeconomic factors that may affect reporting, the spatial and temporal distribution of public rat complaints appeared to be a reliable indicator of rat abundance across community areas in Chicago. This suggests that public complaints may be an effective tool to identify areas with a high likelihood of rat infestations and where rodent control is most needed. This can increase the efficiency of targeted control programs. In contrast, other potential methods of assessing rat abundance (e.g., systematic surveys performed by rodent professionals) are more labor intensive and costly. While complaints-based systems can be biased in other instances of human-wildlife conflict (Howe et al., 2010; Poessel et al., 2013), there may be less bias in the distribution of rat complaints because public attitudes about rats are more uniformly negative and residents may be more aware of the potential risks associated with rats (Bremner and Park, 2007).

Although complaints appear to be a useful indicator of rat activity, relying on public complaints to direct municipal rodent control is intrinsically a reactive method, failing to address issues that promote infestations. However, because several socioeconomic and landscape factors were associated with rat abundance, these community-level characteristics can also be used to help anticipate rat problems before complaints accumulate. For example, trap success was greater in community areas with proportionately more rental housing, which aligns with higher rates of rat bites on blocks with more rental units in New York City (Childs et al., 1998). Rats may be more abundant in rental properties if tenants or off-site managers are less fastidious about reducing attractants or pest control than owners. As such, city planners seeking to target rat infestations before they arise may benefit from prioritizing areas with a greater abundance of rental units.

At a local scale, food attractants in the form of uncontained garbage was the best predictor of both complaints and rat abundance, supporting public education campaigns in Chicago and other cities to "not feed the rats" (Calder, 2018). Such ephemeral attractants are difficult to quantify but may be important for variation in rat problems within community areas. For example, big-data approaches to predicting local pulses in rat complaints found that garbage-related 311 complaints typically precede spikes in rat complaints within a 7-day window (Thornton, 2013). These findings support the growing body of research that suggests that ecological pest management (e.g., that which reduces access to food resources and harborage) is the most effective and sustainable approach to addressing rat infestations (Colvin and Jackson, 1999). As such, city-wide rat control initiatives must consider strategies not just for rats, but also for underlying issues of waste management.

This positive relationship with uncontained garbage may also explain why trap success was negatively associated with vacant land. While several other studies have observed increased rat activity near vacant buildings (Himsworth et al., 2014; Rael et al.,

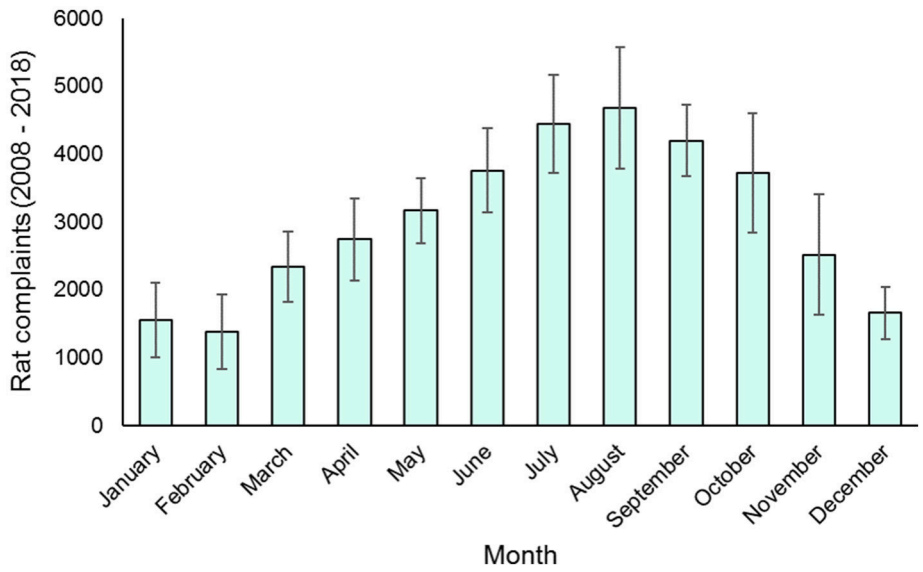

FIGURE 5 | Monthly distribution of rat complaints made to the city of Chicago from April 1 2008 to April 1 2018. Columns show mean values and error bars show standard deviation.

2016), vacant lots may offer little resources in the way of human food waste. There is increasing appreciation for the ecological value of urban vacant lots (Anderson and Minor, 2017) and their role as rat habitat is an interesting avenue for future research.

Our study demonstrates that public rat complaints are a valuable tool to mitigate property damage and public health concerns from rats. However, it is important to note that it is often difficult to determine whether reporting accurately reflects animal abundance. For example, we witnessed an increase in rat complaints in late summer which is consistent with the period when rat recruitment leads to peak rat population density (Feng and Himsworth, 2014). However, this trend may also be driven in part by favorable weather conditions increasing human outdoor activity.

Future studies that aim to understand the situations in which residents report rat complaints, and their motivations behind reporting, will improve our predictions of spatiotemporal patterns in rat complaints and interpreting the causes behind them (e.g., property damage, concerns about disease, pets). Further, in our study, trap success was an efficient method to measure relative abundance while making use of on-going rodent control across a large and diverse geographic area, which has been a challenge in studying urban rat ecology (Parsons et al., 2017). However, these methods are limited to understanding only the trappable rat population, as there may be many individuals that never enter traps. Other approaches, such as mark-recapture (Desvars-Larrive et al., 2018) or population genetics (Combs et al., 2018b) may help elucidate rat population size and genetic structure to evaluate or target rodent control. For example, a better understanding of the processes driving rat abundance across spatial scales will be useful in understanding the impacts of rodent control on the broader population (Gardner-Santana et al., 2009). At even larger scales, future studies examining variation in correlates of rat abundance across cities (Combs et al., 2018a) will improve our ability to generalize patterns of rat infestations.

CONCLUSIONS

In summary, our study reveals that public complaints about rats may be a useful tool to identify areas and time periods with relatively high rat abundance. Further, we demonstrate that prioritizing areas with greater numbers of rental units and uncontained garbage may increase the efficiency of rodent control programs. These approaches require a better understanding of the motivations behind residents' complaints and the relationships between rat abundance and risk of property damage and pathogen transmission. To move beyond reactive approaches to rats, this information can be used to facilitate proactive control techniques (e.g., reducing access to food sources) in many cities around the world.

ETHICS STATEMENT

The study used rat trapping data from ongoing rodent control by a private pest management company and so was exempt from animal care and use committee approval.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher. Requests to access the datasets should be directed to Maureen H. Murray, maureenmurray@lpzoo.org.

AUTHOR CONTRIBUTIONS

MHM designed the study with assistance from KB, RF, MF, MR, and SM. RF oversaw rat trapping and MHM, MR, MPM performed field work. MHM and MF conducted statistical analysis. MHM wrote the paper with assistance from all authors.

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REFERENCES

Anderson, E. C., and Minor, E. S. (2017). Vacant lots: an underexplored resource for ecological and social benefits in cities. *Urban. Urban Green* 21, 146–152. doi: 10.1016/j.ufug.2016.11.015

Ayral, F., Artois, J., Zilber, A. L., Widén, F., Pounder, K. C., Aubert, D., et al. (2015). The relationship between socioeconomic indices and potentially zoonotic pathogens carried by wild Norway rats: a survey in Rhône, France (2010-2012). *Epidemiol. Infect.* 143, 586–599. doi: 10.1017/S0950268814001137

Ayyad, C., Mateu, J., and Tamayo-Uria, I. (2018). Non-linear spatial modeling of rat sightings in relation to urban multi-source foci. *J. Infect. Public Health* 11, 667–676. doi: 10.1016/j.jiph.2018.05.009

Bachelder, A. E., Stewart, M. K., Felix, H. C., and Sealy, N. (2016). Health complaints associated with poor rental housing conditions in Arkansas: the only state without a landlord's implied warranty of habitability. *Front. Public Health* 4:263. doi: 10.3389/fpubh.2016.00263

Bremner, A., and Park, K. (2007). Public attitudes to the management of invasive non-native species in Scotland. *Biol. Conserv.* 139, 306–314. doi: 10.1016/j.biocon.2007.07.005

Calder, R. (2018). *Lousy Anti-Rat Campaign Reminds New Yorkers Not to Feed Rodents*. New York, NY. Available online at: https://nypost.com/2018/05/13/lousy-anti-rat-campaign-reminds-new-yorkers-not-to-feed-rodents/.

Cherone, H. (2017). *Chicago Is The Rat Capital Of The Country, At Least According To Orkin*. dnainfo. Available online at: https://www.dnainfo.com/chicago/

20171016/logan-square/worst-city-for-rats-orkin-rattiest-pest-control-poison.

Childs, J. E., McLafferty, S. L., Sadek, R., Miller, G. L., Khan, A. S., Randy DuPree, E., et al. (1998). Epidemiology of rodent bites and prediction of rat infestation in New York City. *Am. J. Epidemiol.* 148, 78–87. doi: 10.1093/oxfordjournals.aje.a009563

City of Chicago (2010). *Chicago Ward, Community Area and Neighborhood Maps*. Available online at: https://www.cityofchicago.org/city/en/depts/doit/supp_info/citywide_maps.html (Accessed August 10, 2018).

CMAP (2017). *Community Data Snapshots.* Available online at: http://www.cmap.illinois.gov/data/community-snapshots [Accessed February 15, 2018].

Colvin, B. A., and Jackson, W. B. (1999). "Urban rodent control programs for the 21st Century," in *Ecologically-Based Rodent Management*, eds. G. R. Singleton, L. A. Hinds, H. Liers, and Z. Zhang (Canberra, Australia: Australian Centre for International Agricultural Research), 243–258.

Combs, M., Byers, K. A., Ghersi, B. M., Blum, M. J., Caccone, A., Costa, F., et al. (2018a). Urban rat races: spatial population genomics of brown rats (*Rattus norvegicus*) compared across multiple cities. *Proc. R. Soc. B Biol. Sci.* 285:20180245. doi: 10.1098/rspb.2018.0245

Combs, M., Puckett, E. E., Richardson, J., Mims, D., and Munshi-South, J. (2018b). Spatial population genomics of the brown rat (*Rattus norvegicus*) in New York City. *Mol. Ecol.* 27, 83–98. doi: 10.1111/mec.14437

Desvars-Larrive, A., Baldi, M., Walter, T., Zink, R., and Walzer, C. (2018). Brown rats (*Rattus norvegicus*) in urban ecosystems: are the constraints related to fieldwork a limit to their study? *Urban Ecosyst.* 21, 951–964. doi: 10.1007/s11252-018-0772-8

Feng, A. Y. T., and Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and Rattus rattus). *Urban Ecosyst.* 17, 149–162. doi: 10.1007/s11252-013-0305-4

Gardner-Santana, L. C., Norris, D. E., Fornadel, C. M., Hinson, E. R., Klein, S. L., and Glass, G. E. (2009). Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*). *Mol. Ecol.* 18, 2766–2778. doi: 10.1021/nn300902w.Release

Himsworth, C. G., Bidulka, J., Parsons, K. L., Feng, A. Y. T., Tang, P., Jardine, C. M., et al. (2013). Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. *PLoS ONE* 7:e2270. doi: 10.1371/journal.pntd.0002270

Himsworth, C. G., Parsons, K. L., Feng, A. Y. T., Kerr, T., Jardine, C. M., and Patrick, D. M. (2014). A mixed methods approach to exploring the relationship between Norway rat (*Rattus norvegicus*) abundance and features of the urban environment in an inner-city neighborhood of Vancouver, Canada. *PLoS ONE* 9:e97776. doi: 10.1371/journal.pone.0097776

Howe, E. J., Obbard, M. E., Black, R., and Wall, L. L. (2010). Do public complaints reflect trends in human-bear conflict? *Ursus* 21, 131–142. doi: 10.2192/09GR013.1

Hume, K., Terranova, D., and Thomas, C. (2002). Complaints and annoyance caused by aircraft operations: temporal patterns and individual bias. *Noise Health* 4, 45–55.

Johnson, S., Bragdon, C., Olson, C., Merlino, M., and Bonaparte, S. (2016). Characteristics of the built environment and the presence of the Norway rat in New York City: results from a neighborhood rat surveillance program, 2008 – 2010. *J. Environ. Health* 78, 22–29.

Lund, U., and Agostinelli, C. (2018). *CircStats: Circular Statistics, from "Topics in Circular Statistics"* (2001). Available online at: https://cran.r-project.org/package=CircStats

National Oceanic and Atmospheric Administration (2015). *National Weather Service Internet Services Team*. Chicago, IL; Rockford, IL. Available online at: http://www.weather.gov/lot/ord_rfd_monthly_yearly_normals (Accessed August 10, 2018).

Norton, T. W. (1987). The effect of trap placement on trapping success of *Rattus lutreolus velutinus* (Thomas) (Muridae: Rodentia) in North-East Tasmania. *Aust. Wildl. Res.* 14, 305–310. doi: 10.1071/WR9870305

Panti-May, J. A., Carvalho-Pereira, T. S. A., Serrano, S., Pedra, G. G., Taylor, J., Pertile, A. C., et al. (2016). A two-year ecological study of Norway rats (*Rattus norvegicus*) in a Brazilian urban slum. *PLoS ONE* 11:e0152511. doi: 10.1371/journal.pone.0152511

Parsons, M. H., Banks, P. B., Deutsch, M. A., Corrigan, R. F., and Munshi-South, J. (2017). Trends in urban rat ecology: a framework to define the prevailing knowledge gaps and incentives for academia, pest management professionals (PMPs) and public health agencies to participate. *J. Urban Ecol.* 3, 1–8. doi: 10.1093/jue/jux005

Perkins, K., and Sampson, R. J. (2015). Compounded deprivation in the transition to adulthood: The intersection of racial and economic inequality among Chicagoans, 1995-2013. *J. Soc. Sci. Russell Sage Found* 37–54. doi: 10.7758/RSF.2015.1.1.03

Pimentel, D. (2007). "Environmental and economic costs of vertebrate species invasions into the United States," in *Managing Vertebrate Invasive Species: Proceedings* of an *International Symposium* (Fort Collins, CO), 1–8.

Poessel, S. A., Breck, S. W., Teel, T. L., Shwiff, S., Crooks, K. R., and Angeloni, L. (2013). Patterns of human–coyote conflicts in the denver metropolitan area. *J. Wildl. Manage.* 77, 297–305. doi: 10.1002/jwmg.454

Rael, R. C., Peterson, A. C., Ghersi, B. M., Childs, J., and Blum, M. J. (2016). Disturbance, reassembly, and disease risk in socioecological systems. *Ecohealth* 13, 450–455. doi: 10.1007/s10393-016-1157-1

Renthop (2018). *Chicago's Rat Complaints Continue to Grow. Chicago, IL, USA* Available online at: https://www.renthop.com/studies/chicago/chicago-rat-complaints-continue-to-grow#chicagoratsassociation.

Richardson, J. L., Burak, M. K., Hernandez, C., Shirvell, J. M., Mariani, C., Carvalho-Pereira, T. S. A., et al. (2017). Using fine-scale spatial genetics of Norway rats to improve control efforts and reduce leptospirosis risk in urban slum environments. *Evol. Appl.* 10, 323–337. doi: 10.1111/eva.12449

Specialty Consultants (2016). *A Strategic Analysis of the U.S.* Jacksonville, FL: Structural Pest Control Industry.

Thornton, S. (2013). *Using Predictive Analytics to Combat Rodents in Chicago. Gov. Technol.* Available online at: http://www.govtech.com/data/Using-Predictive-Analytics-to-Combat-Rodents-in-Chicago.html.

United States Census Bureau (2016). *QuickFacts.* Chicago, IL. Available online at: https://www.census.gov/quickfacts/chicagocityillinois (Accessed August 10, 2018).

Walsh, M. G. (2014). Rat sightings in New York City are associated with neighborhood sociodemographics, housing characteristics, and proximity to open public space. *PeerJ* 2:e533. doi: 10.7717/peerj.533

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SYSTEMATIC REVIEW
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Rats About Town: A Systematic Review of Rat Movement in Urban Ecosystems

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Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are ubiquitous urban pests, inhabiting cities worldwide. Despite their close association with people, urban rats remain difficult to control. This can be partly attributed to a general lack of information on basic rat ecology to inform management efforts. In this systematic review and narrative synthesis, we collate the published literature to provide a comprehensive description of what is known about urban rat movement, including information on home range, site fidelity, dispersal, movement patterns, barriers to, and factors impacting, movement. We also discuss the methodologies used to track and infer rat movement, as well as the advantages and limitations of employing these techniques. Our review suggests that the distances traveled by urban rats are location-specific, determined by both local resource availability and barriers to movement such as roadways. Although roads may impede rat movement, genetic techniques suggest that rats traverse roadways more often than revealed by capture-based tools, while long-distance dispersal events by either natural migration or facilitated by humans (i.e., as stowaways in transport vehicles) can maintain connectivity among distant populations. Because rat movement patterns are related to the transmission of rat-associated pathogens and the success of rodent control programs, these results have implications for city planners, pest control efforts, and public health. Therefore, we emphasize the importance of understanding local rat movement patterns in order to devise and deploy efficient and effective rat mitigation initiatives in urban centers.

Keywords: dispersal, ecology, home range, immigration, movement, rat, *rattus*, urban

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INTRODUCTION

The presence of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) is an important and growing issue in cities globally due to their associated health and economic impacts (Feng and Himsworth, 2014). For example, rats pose a risk to public health as they are the source of a variety of zoonotic pathogens (disease-causing microbes transmissible from rats to people, e.g., *Leptospira interrogans*) responsible for significant human morbidity and mortality (Himsworth et al., 2013b). Infestations can also serve as a chronic stressor, impacting both the mental and physical health of residents (German and Latkin, 2016; Lam et al., 2018). Rats also damage urban infrastructure (due

to chewing and burrowing activities) and contaminate foodstuffs. Finally, infestations can result in substantial economic losses, both directly (i.e., costs associated with rat control), and indirectly (i.e., costs associated with mitigating and repairing rat-associated damage) (Pimentel et al., 2005; Almeida et al., 2013). Given rapid urbanization, these issues are likely to increase in future; 55% of the world's population resides in cities, with a projected increase to 68% by 2050. Much of this growth will occur in developing regions (United Nations, 2018) where rat-associated risks are higher due to issues of inadequate housing, infrastructure, and sanitation (Himsworth et al., 2013b). Further, a lack of effective tools to address rat infestations (Parsons et al., 2017) underscores the importance of re-thinking our current approaches to rat control.

To monitor, and mitigate the impact of rats, an understanding of their spatial ecology is paramount. For example, the extent to which animals move within and utilize the environment dictates both the epidemiology of the pathogens they carry (Volkova et al., 2010; Guivier et al., 2011; Quixabeira-Santos et al., 2011) as well as the scale at which pest control efforts will have the greatest success (Bomford and O'Brien, 1995; Robertson and Gemmell, 2004; Adams et al., 2014). One of the most significant remaining knowledge gaps relevant to describing the spatial ecology of urban rats is information regarding the extent of their home range (Desvars-Larrive et al., 2018). The home range represents the area frequented by an individual (Davis et al., 1948) and typically encompasses areas used for food acquisition, mating, and rearing young (Burt, 1943). However, as individuals differentially use areas of their home range according to factors such as age, sex, population density, season, and environmental variability (Wolff, 1985; Cederlund and Sand, 1994; Wiktander et al., 2001; Dahle and Swenson, 2003; Kjellander et al., 2004; Börger et al., 2006; Safi et al., 2007), or may even migrate to new home ranges (Burt, 1943), information on home range size alone may underestimate the true area traversed by rats.

Beyond home range size, spatial ecology requires an understanding of the detailed movements of rats within cities. This includes information on dispersal distances (i.e., movement away from the natal or home area) (Drickamer, 1987), and how features of, or changes to, the urban environment impact movement. For example, a meta-analysis found that terrestrial mammals residing in cities traveled shorter distances than did their non-urban counterparts (Tucker et al., 2018). This reduced structural connectivity may result from the varied quality and size of habitat patches in cities (Dickman and Doncaster, 1987), as well as the physical barriers posed by roadways (Rondinini and Doncaster, 2002). However, as rats can occupy habitats with a diverse set of characteristics (Himsworth et al., 2014a) they may exhibit greater structural connectivity than other urban wildlife.

The objective of this review is to summarize, compare, and evaluate the published literature detailing the movement patterns of urban Norway and black rats. We describe the tools that have been used for studying urban rat movement as well as the challenges of employing these techniques. Finally, we describe how information regarding the spatial ecology of rats may be of relevance to different stakeholders and identify remaining gaps in knowledge to be addressed in future ecological research.

Abbreviations: CMR, capture-mark-recapture.

METHODS

Search Strategy

From May 2018–July 2018 we performed systematic searches following the approach outlined by Moher et al. (2009). Our search included the databases: Web of Science CORE, CAB Direct, JSTOR, Medline, and Zoological Record. We used keyword combinations pertaining to the following concepts: Rats (*Rattus norvegicus,*" "Norway rat*," "brown rat*," "*Rattus rattus,*" "black rat*," "roof rat*"), movement (dispersal, emigration, expansion, immigration, migration, movement, boundaries, distribution, domain, "home range*," "home area*," "site fidelity," territory, zone) and the urban environment (urban, city, cities, municipal, suburban, residential, metropolis, metropolitan). The groups of keywords within each concept were combined using the Boolean operator "OR" and concepts were combined using "AND" (see **Supplementary Table 1**). We included literature from the earliest cut-off date for each database.

Study Selection

Titles and abstracts were screened for eligibility using the abstract screener function in METAGEAR (Lajeunesse, 2016). This package presents paper titles and abstracts in a graphical user interface for reviewer coding for inclusion or exclusion. Thirty percent of papers were screened by two authors (KAB and MJL) to ensure screening consistency. Articles deemed eligible in the first round of screening were reviewed in full by both KAB and MJL. Papers were excluded if they focused on rural rat populations, global rat migration patterns, or did not measure aspects of rat movement (either directly or indirectly). Literature in languages other than English were excluded. Additional sources were added through citation searching.

Data Collection and Analysis

Included papers were grouped by trapping methodology (i.e., continuous tracking, capture-mark-recapture, genetic techniques, and proxy methods). The content of each paper was summarized using a matrix method (Garrard, 2013) in which a number of categories relevant to describing the study characteristics (i.e., study location, study scale, species studied, sample size, methods used) and rat movement (i.e., home range, dispersal, areas and extent of movement, factors impacting movement, other relevant findings) were determined *a priori*. Each paper was reviewed and summarized according to these categories, and we compared information within each category across studies. Findings were synthesized using a narrative synthesis methodology which involves summarizing the findings of multiple works in text format (Arai et al., 2007). The following synthesis pertains to Norway and black rat movement patterns in urban ecosystems and is reviewed within six themes derived during the synthesis: study design, home range, site fidelity, dispersal, movement patterns, barriers to movement, and factors impacting movement.

RESULTS

Study Selection and Characteristics

Our initial search resulted in 1665 sources, 105 of which were reviewed in full (**Figure 1**). Of the final group of 39 papers, two were extensions of other included studies that contained additional relevant information. Therefore, we reviewed 37 unique studies examining the movements of Norway rats ($n = 30$), black rats ($n = 6$), or both ($n = 1$).

Although published research on rat movement occurs as early as 1915, nearly half (48.6%; $n = 18$) of included studies were published in the past decade (**Figure 2**), and approximately half (51.4%; $n = 19$) were conducted in North America (**Supplementary Table 2**). See **Supplementary Table 3** for details of the included studies.

Study Design

In general, all studies sought to describe urban rat ecology, but most (56.8%; $n = 21$) explicitly mentioned using this information to inform pest control. Both direct and indirect methods were employed in the study of rat movement (**Figure 2**). Direct measures included Capture-Mark-Recapture (CMR; $n = 12$) and continuous tracking (e.g., direct observation, radio-tracking, and Global Positioning Systems (GPS); $n = 7$). Indirectly, movement was assessed through proxy measures of rat movement (e.g., track pads, rat tracks in snowfall, bait uptake, and feces marked with bait-specific dye; $n = 11$) and population genetics-based techniques ($n = 11$). In some instances ($n = 3$), multiple methods were employed. See **Box 1** for an overview of these tools.

Home Range

For both Norway and black rats (hereafter termed "rats" when referencing both species), the home range size and shape is determined by access to feeding and harborage sites (Davis et al., 1948; Recht, 1982; Low et al., 2013) as well as access to mates (Low et al., 2013; Glass et al., 2016). These associations lead to irregularly shaped home ranges with individuals often moving along narrow pathways connecting harborage and food sources (Davis et al., 1948; Recht, 1982; Recht et al., 1983). The presence of conspecifics may also influence home range size, as some individuals have been found to avoid the home ranges of other

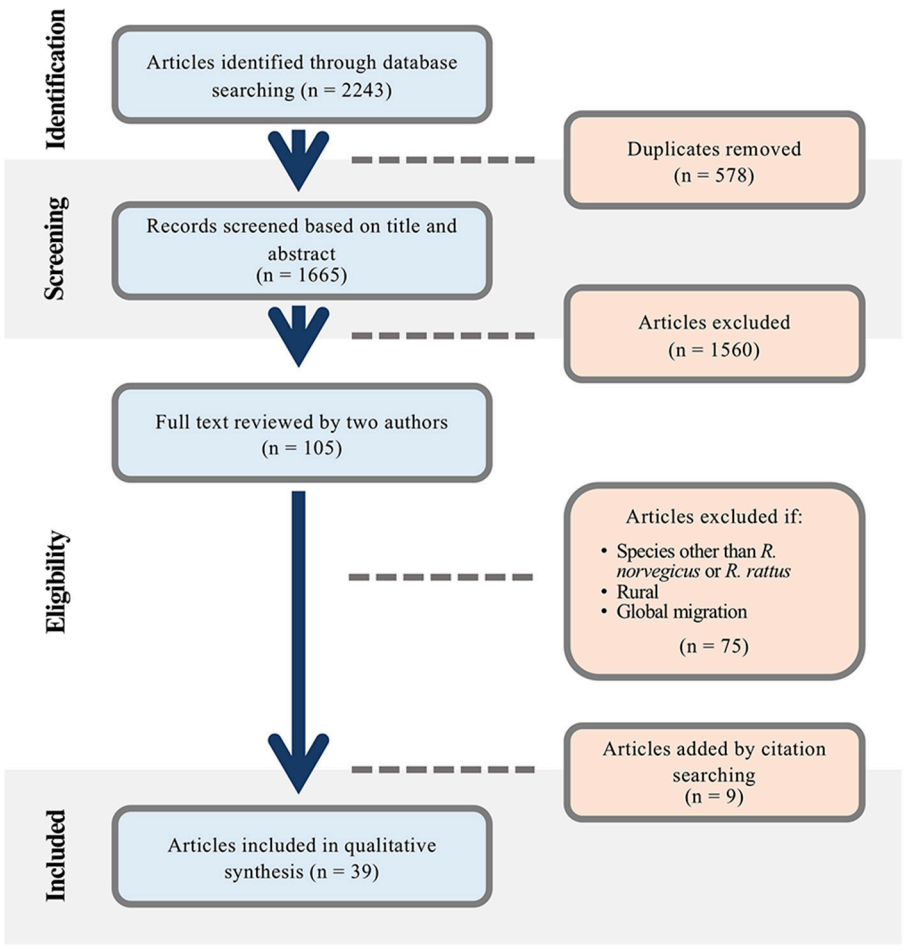

FIGURE 1 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart depicting the screening process of articles included in the narrative synthesis.

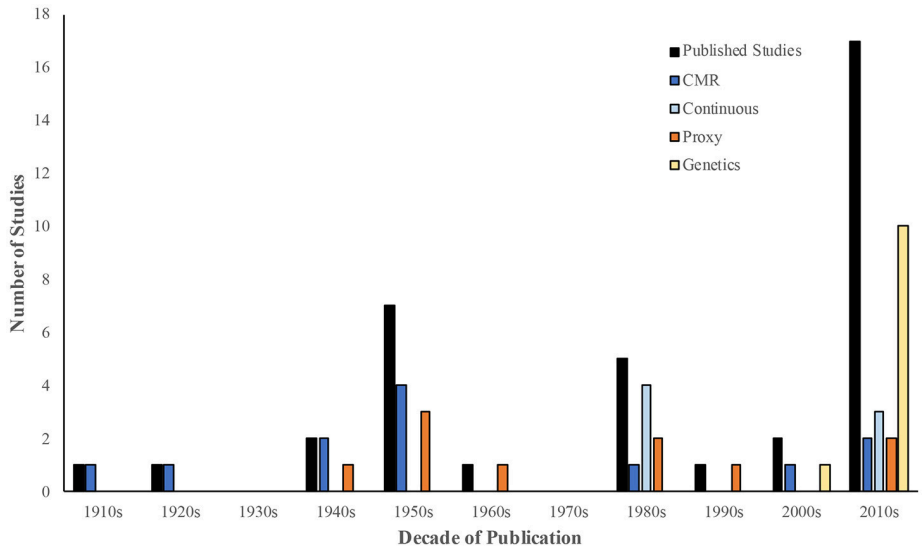

FIGURE 2 | The number of unique published studies ($n = 37$) included in the review by decade of publication. Within each decade, the number of studies employing direct (Capture-Mark-Recapture (CMR) and continuous tracking), and indirect methods (proxy measures and genetics-based tools) to assess rat movement are indicated.

BOX 1 | An overview of methods commonly used for studying urban rat movement, their benefits, and limitations.

Direct Measures: To quantify the movements of urban rats, many studies rely on trapping-based techniques. Capture-Mark-Recapture (CMR) methods involve trapping and tagging individuals (e.g., with numbered ear tags or Passive Integrated Transponder (PIT) tags) for future identification. Following tagging, animals are released at their original point of capture and are later re-caught. Movement is determined by measuring the distances between the traps in which rats are caught, or in the case of PIT tags, by the distances between sensor stations through which rats pass (Parsons et al., 2015). In this way, PIT tags are advantageous as they decrease handling-time of rats. Although these tools allow researchers to track large numbers of rats (e.g., 341 in Petrie and Todd (1923), they are labor-intensive, rely heavily on trap placement, and yield fragmented data as they do not account for movement between capture points (Hayne, 1949; Glass et al., 2016). Moreover, these techniques are hindered by the neophobic nature of rats, which can result in low recapture rates and trapping bias (Barnett, 1963; Tanaka, 1963; Taylor, 1978).

To derive more complete descriptions of movement patterns, researchers have directly observed the behaviors of individual rats (Calhoun, 1963; Takahashi and Lore, 1980; Glass et al., 1989); but, this strategy is limited by the number of individuals which can be tracked at one time and is difficult when animals are not readily visible (Takahashi and Lore, 1980). Two tools that combine trapping and observation are Very-High-Frequency (VHF) radio-telemetry and Global Positioning System (GPS) technologies which require affixing rats with tags that transmit movement data in real-time (VHF), or store location data in the tag for either future retrieval or remote download by the observer (GPS). Both VHF radio-telemetry and GPS-based tools allow for improved spatial resolution of rat movements, but until recently they have been hampered by tag size which is limited to 5% of the animal's weight to minimize negative tag-associated effects (Animal Care Use Committee, 1998). While sample sizes for both methods tend to be smaller than for CMR (Tomkiewicz et al., 2010), GPS-based tools offer advantages over VHF radio-telemetry which requires close-range observation of the animal, potentially influencing natural behaviors (Cooke et al., 2004; Parsons et al., 2014). Further, radio-telemetry is challenging in cities where buildings and other structures can interfere with radio signals (LaPoint et al., 2015). Yet, while GPS-based tools may be more beneficial for these reasons, they remain difficult to deploy on urban rats due to issues of tag removal and tag-satellite line-of-sight obstruction (Byers et al., 2017). Moreover, the costs of GPS tags over radio-telemetry may make this method prohibitive (Cagnacci et al., 2010).

Indirect Measures: Rat movements can also be inferred using indirect measures. Visibly observing the tracks made by rats (e.g., in snow) allows for estimates of space use (Davis et al., 1948), but is naturally limited by the availability of snow. When the environment is not suitable for observing tracks, researchers can deploy track plates which become marked with characteristic "rat signs" when rats travel across them (Hacker et al., 2016). Rat movement may also be determined through bait consumption. This method is common among studies assessing re-infestation of areas where rat eradication efforts were previously enacted (Barnett et al., 1951; Barnett and Bathard, 1953; Greaves et al., 1968; Andrews and Belknap, 1983; Colvin et al., 1998). Bait that is dyed with a compound that colors rats' feces can also be used to calculate the extent of space use around dyed-bait stations (Davis et al., 1948). While these methods are less time consuming to enact than the direct measures previously mentioned, they provide only minimal information on rat activity.

Recently, there has been an increase in the use of population genetics-based methods to infer rat movement (see **Figure 2**). These tools analyze differences among individuals at specific locations of the genome caused by genetic mutations. These mutations can result in single nucleotide polymorphisms (i.e., SNPs) (Richardson et al., 2017; Combs et al., 2018a,b) or rearrangements resulting in different numbers of small repeating sequences (e.g., microsatellites) (Gardner-Santana et al., 2009; Kajdacsi et al., 2013). Using these tools, researchers can infer historical movements by identifying the distances between relatives (e.g., parents and offspring; Costa et al., 2016; Glass et al., 2016), and identifying potential migrants (e.g., individuals genetically assigned to a population other than the one in which they were caught; Kajdacsi et al., 2013; Berthier et al., 2016; Desvars-Larrive et al., 2017). These methods have the benefit of supporting large sample sizes (e.g., 1220 in Combs et al., 2018a), but they are limited to detecting first-generation migrants and movements during which rats mate and are reproductively successful, underestimating true levels of connectivity among populations (Richardson et al., 2017).

rats (Low et al., 2013; Oyedele et al., 2015). Beyond the value of regular access to resources, an intimate familiarity with the features of the home range may serve as a protective measure for rats. For example, individuals within their home range have been recorded entering areas of cover (e.g., a rat hole) more rapidly than those in areas outside of their home range (Davis et al., 1948). This is further evidenced by rats' neophobic behaviors toward new and/or introduced features such as traps (Barnett, 1963).

Rats are familiar with the extent of their home range (Recht, 1982; Recht et al., 1983), but usage is concentrated within a fraction of this area (Low et al., 2013; Oyedele et al., 2015). Termed the "core home range," this region represents the space where an animal spends 50% of its time (Downs and Horner, 2008). The two studies which calculated core home range size for urban rats estimated its size as 11% of the total home range for Norway rats (Oyedele et al., 2015), and approximately 31% of the space used for black rats (Low et al., 2013). Studies in both species have found that the core home range encompasses important food sources, the home burrow, and areas of dense vegetation (Davis et al., 1948; Recht, 1982; Recht et al., 1983). However, rats will travel throughout the home range to occupy alternate burrows, particularly when their home burrow is disrupted (Recht, 1982; Recht et al., 1983). In fact, rats have been found to switch the location and extent of their home range altogether (Davis et al., 1948; Low et al., 2013), signifying that the size, shape, usage, and location of the home range are flexible for individual rats and dependent on physical and environmental characteristics.

Home range size has also been found to vary by sex, with evidence that male Norway rats occupy larger home ranges than do females (Tanaka and Kawashima, 1951; Oyedele et al., 2015). This may be due to differences in reproductive behavior, whereby males increase their ranges to actively search for mates (Dowding and Murphy, 1994). For example, in Norway rats, the area of the total and the core home range of males was approximately 13X and 5X larger than that of females, respectively (Oyedele et al., 2015). In black rats, the total and core home range area for males was 4X and 3.5X greater than for females, respectively (Low et al., 2013). Indeed, home ranges of male black rats have been shown to overlap with those of other males and females, whereas females had home ranges that were exclusive of each other (Low et al., 2013), further supporting the role of mate-searching in determining the extent of the home range.

Studies also indicate that home range size and shape vary by location (Davis et al., 1948; Recht, 1982; Recht et al., 1983; Oyedele et al., 2015). For example, the home range for Norway rats in Baltimore, Maryland was 30–45 m in diameter (Davis et al., 1948), and in George Town, Malaysia the average home range size was 130 m^2 (Oyedele et al., 2015). Similarly, for black rats, home range size did not exceed 30.5 m in diameter in the City of Orange, California (Recht et al., 1983) while on Christmas Island it was 5330 m^2 (Low et al., 2013). As home ranges of urban rats are irregularly shaped (Davis et al., 1948; Recht, 1982; Recht et al., 1983), and because home range estimates will vary depending on the methodology used (see **Box 1**) it is impossible to directly compare these measurements. However, studies in both Norway and black rats have suggested that differences in home range size between study sites may be due to differences in resource availability (Low et al., 2013; Oyedele et al., 2015).

Site Fidelity

Distances traveled by rats are dependent on the presence of harborage and food in the environment (Creel, 1915; Petrie and Todd, 1923; Davis et al., 1948; Heiberg et al., 2012). When these are readily available, rats display a strong site fidelity, rarely leaving their home area. For example, CMR studies in Norway rats have found that 27–63% of rats in residential areas were recaught in the same location as their prior capture (King, 1950; Tanaka and Kawashima, 1951; Glass et al., 1989) although this was less common (e.g., 8% of rats) in urban parklands (Glass et al., 1989). Genetic results support these findings. For example, by DNA fingerprinting methods 95% (Gardner-Santana et al., 2009) and 97% (Glass et al., 2016) of rats were genetically assigned to the area of their capture (i.e., based on genetic similarity to other rats in the vicinity of their capture, they were more likely to have been born in the area in which they were caught than in another sampled site). Strong site fidelity was also revealed by Costa et al. (2016), who genotyped male rats as well as pregnant females and their offspring, and found that males with a high probability of siring offspring (>99%) were within 70 ± 58 m of the pregnant female. Similarly, Richardson et al. (2017) demonstrated that rat movement occurred mostly within the area (i.e., valley) from which rats were sampled. Estimates for site fidelity in urban black rats have not been documented.

Daily movements by rats are typically over short distances. For Norway rats, various CMR studies have documented typical movements ranging from 10 to 20 m (Davis et al., 1948; Tanaka and Kawashima, 1951; Glass et al., 1989; Parsons et al., 2015). In urban parklands, Norway rats have been recorded moving greater average distances of 25 m (Glass et al., 1989). In comparison to CMR, genetic analyses have demonstrated that Norway rats move further still (e.g., 30–150 m), approximately corresponding to the length of a city block (Gardner-Santana et al., 2009; Combs et al., 2018b). Interestingly, limited movement was supported by an analysis of the ectoparasite communities of urban Norway rats. In this study, Angley et al. (2018) found that rats located near each other geographically had more similar assemblages of ectoparasites than did rats located further apart. Because rat ectoparasites are transmitted among individuals via close contact, this implied that rats near each other came into contact with each other more frequently than those further apart.

In urban sewer systems, rats have been found to travel further distances day-to-day than their above ground counterparts. For example, in London, England sewer rates moved up to 77 m (Bentley et al., 1958) while in Copenhagen, Denmark, rats traveled up to 200 m in a day (Heiberg et al., 2012), over 10X the distance recorded for surface populations. In this way, sewers may be more easily traversable, serving as conduits to movement. Interestingly, while daily distances traveled by rats are thought to be greater for males than females (Davis et al., 1948), this does not appear to be the case for sewer populations (Heiberg et al., 2012), suggesting that the environment is a strong determinant of distances traveled.

Dispersal

Dispersal of rats away from their natal site is generally over short distances. For example, mean dispersal distances between parents and offspring have been recorded as 45 m for Norway rats (Combs et al., 2018b) and 496 m for black rats (Mangombi et al., 2016) while distances between putative parents (dams and sires of offspring) ranged from 0 to 353 m for Norway rats (Costa et al., 2016; Glass et al., 2016). Genetic patterns of isolation by distance (whereby individuals are more closely related to rats in neighboring areas than they are to individuals further away) corroborate these trends (Gardner-Santana et al., 2009; Mangombi et al., 2016; Combs et al., 2018a,b). For example, in a multi-city comparison of Norway rat population genetic structures, rats were generally highly related to each other at distances within 500 m (Combs et al., 2018a). However, evidence for isolation by distance has not been found by all studies (Kajdacsi et al., 2013; Berthier et al., 2016).

Less often, dispersal can occur over long distances. For example, dispersal distances have been recorded up to 11.5 km for Norway rats (Gardner-Santana et al., 2009). Although such extended movements are infrequent [e.g., 19 of 230 black rats (8.2%) were classified as migrants in Sahel Niger (Berthier et al., 2016)], evidence of gene flow among Norway rat colonies from 1.5 to 3 km apart suggests that connectivity among populations is maintained by immigration amongst colonies (Gardner-Santana et al., 2009; Combs et al., 2018a). This dispersal may be non-random, whereby individuals move among similar habitat types (e.g., residential areas vs. mixed-used; Angley et al., 2018) and may also be facilitated anthropogenically, such as by commercial transport along road networks (Berthier et al., 2016).

For rats, dispersal has been primarily associated with resource availability and competition, dominance hierarchies, and mating behavior (Calhoun, 1963; Glass et al., 1989). Specifically, when feeding and harborage sites are scare, rats may travel significant distances in search of resources. For example, when in an unfamiliar resource-poor area, Norway rats have been recorded traversing twice the distance as individuals in unfamiliar resource-rich locations (6 vs. 3 km; Creel, 1915). Mate-searching is also an important driver of dispersal, with rats (particularly males) extending their movements in search of mates (Davis et al., 1948; King, 1950; Glass et al., 2016). Sex-biased dispersal has been documented in Norway rats where the majority of migrants are often reproductively mature males (Gardner-Santana et al., 2009; Kajdacsi et al., 2013; Desvars-Larrive et al., 2017). Sex-biased dispersal has been further evidenced by close proximity among related females caught at a fine spatial scale, suggesting that females moved shorter distances than males in the same population (Desvars-Larrive et al., 2017). While these patterns have not been observed in all studies (Gardner-Santana et al., 2009; Combs et al., 2018b) they align with foundational experimental research on Norway rats that found that mature male rats dispersed greater distances than adult females and juveniles (Calhoun, 1963).

In cases where rats immigrate into stable populations, invading rats may be unable to successfully establish home ranges, necessitating extended movements of evicted rats. For instance, the introduction of 112 foreign rats into a city block resulted in the invaders being more likely to emigrate from the site of release than were resident rats in the same area (Calhoun, 1948). Further, the immigration of large numbers of rats into a population may temporarily decrease the reproductive rate of the resident population (Davis and Christian, 1956). Therefore, although dispersal can maintain connectivity among populations, not all immigration events are successful, and can, in some cases, disrupt the regular population dynamics of the resident population.

Movement Patterns

Rats are generally found to be nocturnal (Recht, 1982; Recht et al., 1983) with heightened activity 2–3 h before sunrise and after sunset (Takahashi and Lore, 1980; Recht et al., 1983). However, rats may also be active during the day (Recht, 1982). Indeed, Parsons et al. (2015) found that rats were active between 06:00 and 19:00 with declining activity in the late morning/early afternoon. These activity patterns have also been shown to differ between the sexes, with males generally active longer than females (Parsons et al., 2015), leaving their burrow 1–2 h before females living in the same area (Oyedele et al., 2015). However, as rat activity varies by location, and across differing study methodologies, it is unclear how aspects of the environment and study design contribute to these differences in activity patterns.

During times of activity, rats generally traverse the same pathways (Recht, 1982; Recht et al., 1983; Oyedele et al., 2015). However, they may use alternate routes to adapt to environmental change. For instance, Recht (1982) recorded Norway rats using alternate pathways both to obtain food left over from picnickers and to avoid people. Norway rats typically move along the ground through narrow runways (Davis et al., 1948), near to fences and other cover (Glass et al., 1989), while black rats utilize aerial features such as greenery, pipes, and wires (Worth, 1950). Both species have been found to travel between adjacent buildings (Petrie et al., 1924; King, 1950; Tanaka and Kawashima, 1951; Recht, 1982; Recht et al., 1983; Hacker et al., 2016). Indeed, Tanaka and Kawashima (1951) observed rats moving among three to four houses in a city block over the course of a single week. Additionally, rats may travel between surface and sewer locations (Colvin et al., 1998; Heiberg et al., 2012), but not in all cases (Gras et al., 2012). In contrast, rats do not appear to travel between adjacent, but separate, sewer systems (Heiberg et al., 2012).

Barriers to Movement

Landscape features such as roads, waterways, and "resource-deserts" (areas with very limited resources) may impede the movement of rats throughout cities (Combs et al., 2018a). In general, roadways are reported as the most common barrier to rat movement (Petrie et al., 1924; Davis et al., 1948; King, 1950; Worth, 1950; Traweger and Slotta-Bachmayr, 2005; Richardson et al., 2017). This is supported by findings that Norway rat home ranges rarely overlap with roads (Davis et al., 1948), and by few cases of rats moving among city blocks (Petrie and Todd, 1923; Calhoun, 1948; Davis et al., 1948; Emlen et al., 1949; Worth, 1950). For example, of 146 black rats trapped in Egypt, only one moved between city blocks (Petrie and Todd, 1923). Likewise, in

a study which followed Norway rat tracks in fresh snowfall, Davis et al. (1948) estimated the rate of road crossings to vary from one crossing every 66 days to one crossing a day, with the frequency of crossing reliant on resource availability.

The permeability of roadways is dependent on their width. While larger roadways have deterred movement more than smaller roadways (Petrie and Todd, 1923), even the width of an alley may impede rats. For example, Davis et al. (1948) found that almost all dyed feces were located on the same side of the alley as bait stations. Although rats may avoid crossing alleys, they traverse them more frequently than roadways. An observation of rat movement found that rats crossed alleys 80X more often than they crossed roads (Glass et al., 1989). Given that rats may also move greater average distances in underground infrastructure such as sewers (Heiberg et al., 2012), barriers posed by roads may be overcome by alternate means of crossing heavily trafficked spaces.

While CMR studies suggest that movement among city blocks are infrequent, genetic analyses demonstrating gene flow reveal that movement is more frequent (Gardner-Santana et al., 2009; Glass et al., 2016; Mangombi et al., 2016; Combs et al., 2018b). For example, by analyzing the genetics of pregnant females and their offspring, Glass et al. (2016) demonstrated that females mated most often with males trapped in alleys other than their own. The authors suggested that this pattern likely occurred through "mate chases" in which groups of males left their home site to mate with females in neighboring blocks (support for multiple paternity of litters is further supported by Costa et al., 2016). In combination, these results indicate that roads are permeable to rat movement and that movement among blocks may be driven by mate searching (Glass et al., 2016) or resource availability (Davis et al., 1948).

Factors Impacting Movement

Because rat movement patterns are dictated by features of the urban environment, changes in weather and anthropogenic habitat modification alter normal rat movement patterns. For example, Recht (1982) observed that Norway rats cease movement in rain, while black rats continue to forage (Recht et al., 1983). Habitat modification can alter rat movement due to either the removal of areas of harborage and/or by blocking typical movement routes. For example, Recht (1982) found that habitat modification (e.g., trimming of vegetation, and removal of debris) resulted in increased Norway rat activity and exploration, larger home ranges, and movement into previously unvisited areas, while construction caused black rats to move to alternate burrows (Recht et al., 1983).

Control methods (e.g., trapping and poisoning) can also promote rat movement, as individuals migrate to occupy and recolonize previously targeted sites. For example, Kajdacsi et al. (2013) demonstrated that following control efforts, there was both population replacement (i.e., local rat reproduction by surviving rats) and recolonization due to the migration of individuals from surrounding areas. Rat migration may also play a role in the re-infestation of urban sewers systems. For example, following rodent reductions of up to 88% (as indicated by bait uptake), rat populations increased from 3 to 20% per week (Barnett and Bathard, 1953; Bentley et al., 1959; Greaves et al., 1968). This increase was attributed in part to rat immigration from within the same sewer system (Greaves et al., 1968) and from an influx of surface populations (Barnett and Bathard, 1953). Such population rebounds can be rapid, occurring in as little as 4 weeks post-eradication efforts (Hacker et al., 2016).

DISCUSSION

Overall this synthesis highlighted a number of important characteristics of urban rat movement. Specifically, rat movement is dependent on the distribution and availability of important resources such as food and harborage and is limited by the barriers posed by features including roads and waterways. While roads may relegate individuals to home ranges corresponding to the limits of a city block, recent studies suggest that rats cross city streets more often than previously estimated. Further, normal movement patterns may be altered due to environmental change, prompting rats to move greater distances still. These findings have several important implications for city planners, pest control professionals, and public health officials seeking to monitor and mitigate the economic and public health impacts posed by urban rats. However, despite increased scientific attention to describing urban rat ecology in the past decade, this review reveals that the specific details of their spatial ecology remain largely undescribed.

Implications for City Planners

This review illustrates that features of the urban environment influence the spatial ecology of rats. Because city planners determine and design many aspects of cities, they have the potential to create spaces less prone to rat infestation. For example, as features such as urban parkland may be more easily traversed by rats (Glass et al., 1989), approaches to reduce waste and improve infrastructure/building conditions in and around these areas may lower rats' ability to infest surrounding regions. However, while our review suggests that parkland and sewer systems may facilitate rat movement, the specific landscape features and socioeconomic attributes which determine the connectivity of rat populations within cities are still poorly understood (LaPoint et al., 2015). Indeed, the only multi-city comparison of urban rat population structure found that the local environment is a strong determinant of rat movement (Combs et al., 2018a). Therefore, it is becoming increasingly important to identify both common features among cities which influence rat movement as well as local features, which can be used by city planners to target and predict areas prone to rat infestation and re-infestation. Targeting these features is particularly important in under-served and marginalized communities where residents are at heightened risk of exposure to large numbers of rats and their associated health and economic impacts (Himsworth et al., 2013b; Costa et al., 2015b).

Urban centers are continuously expanding and undergoing dramatic habitat modification (Grimm et al., 2008). These issues are enhanced in rapidly urbanizing under-resourced settings (United Nations, 2018) where unplanned urban development and land use changes (e.g., Chitrakar et al., 2016; Pawe and

Saikia, 2018) pose additional challenges. Our review suggests that habitat disturbance can instigate long distance movements by rats; this is particularly relevant to city planners as ubiquitous activities such as demolition and construction may both create an environment suitable for rat harborage (e.g., open soil and shelter from equipment) (Colvin and Jackson, 1999) as well as drive rats from disturbed sites to surrounding areas (Richter, 1968; Battersby et al., 2002). To pre-empt the potential for rat colonization and migration, city planners should consider employing Integrated Pest Management approaches to proactively decrease habitat suitability and migration risks. Integrated Pest Management is a multi-faceted approach which incorporates long-term planning goals, data management, as well as partnership among governments, private pest control companies and communities (Colvin and Jackson, 1999). Such an approach may include implementing policies that require the eradication of rats prior to the demolition of a building to minimize the efflux of rats and the degree of subsequent colonization. This requires coordination among developers and private pest control companies to identify areas for control, enact control efforts, and monitor the success of the control campaign. For regions where development is primarily undertaken by urban dwellers as opposed to municipalities (i.e., in urban slums), city planners might instead focus on educating communities about the importance of undertaking pest control before and during construction, as well as providing resources such as traps or deploying private pest control companies to areas with ongoing development. In tandem, actions to reduce food sources and harborage sites in adjacent city blocks or homes can also decrease their attractiveness to rats and their ability to support rat population growth. These initiatives also require community involvement and educational programs to inform residents about the ways to properly dispose of food waste and remove potential harborage sites (Colvin et al., 1998). Although Integrated Pest Management approaches require sustained and significant investment (i.e., in time and personnel), reactive approaches which fail to address the underlying features that promote rat abundance and facilitate rat movement are likely to remain ineffective.

Implications for Pest Control

It is necessary for control methods to account for rat movement patterns. Studies have demonstrated that control campaigns aimed at culling rats alone can be compromised by rapid population rebounds due to reproduction by surviving rats (Barnett and Bathard, 1953; Hacker et al., 2016), and/or immigration of individuals from surrounding areas (Kajdacsi et al., 2013). Given that rats readily cross roads for resources and mating (Davis et al., 1948; Glass et al., 2016), our review suggests that limiting the scale of control to a single property or city block is likely to be ineffective due to reinvasion of the targeted site. Instead, efforts should focus on effectively identifying and targeting areas at the scale of "eradication units." These areas represent the spatial scale at which rats are interconnected, allowing for recolonization following a control intervention (Abdelkrim et al., 2007). For example, in Salvador, Brazil where the majority of Norway rat movement was found to occur within a valley, targeting rat populations at the level of the valley might be appropriate (Richardson et al., 2017). In contrast, a study evaluating the genetic signatures of black rat populations before and after an eradication campaign on four islets in the French Caribbean, found that control efforts would need to extend to surrounding islands to minimize the potential for re-invasion (Abdelkrim et al., 2007). Because the extent of movement varies by location (Combs et al., 2018a), deriving specific recommendations as to the scale of pest control efforts is difficult. Yet, to design effective control strategies, research that quantifies the contribution of landscape attributes to rat migration is necessary to help pest control professionals define the scale of control and prevention approaches. Further, to support the integration of scientific knowledge into actionable information for pest control professionals, it is necessary that projects evaluate how scaling control efforts to the level of local rat movement (i.e., eradication units) compares to traditional pest control efforts.

An understanding of rat movement is not only necessary for more effectively implementing current pest control practices, but it is also important in developing and deploying future pest control innovations. For example, gene drive technologies have received increasing attention for their potential pest control applications. Gene drive technologies involve genetically engineering individuals so that sets of genes are disseminated within populations through sexual reproduction. For pests, genes which lower the fertility and fecundity of individuals are of particular interest (Moro et al., 2018). Because the spread of these traits throughout a population is reliant on interactions among individuals, understanding local rat movement ecology will be necessary to inform the implementation of these technologies in these species.

Implications for Public Health

Our review supports the long-held position that much of rats' activities remain within the confines of a single city block. Because many of the pathogens carried by rats are transmitted through close contact among conspecifics (Childs et al., 1998; Himsworth et al., 2013b) this limited movement implies that most transmission events are also restricted to within-block populations. These findings support prior research by Himsworth et al. which demonstrated significant heterogeneity in pathogen prevalence across adjacent city blocks (Himsworth et al., 2013a, 2014b, 2015), such that some blocks had many infected rats and other blocks had very few. Similar findings have been demonstrated in Salvador, Brazil, where shedding of the pathogen *L. interrogans* by Norway rats varied significantly by location (Costa et al., 2015b). If limited movement allows for the clustering of pathogens, then the risk of encountering an infected rat may be site specific. In tandem with these results, our review suggests that activities that disrupt rat colonies could increase movement within and between blocks. Evidence suggests that when rats migrate to surrounding colonies, they fight (Calhoun, 1948), and as aggressive behaviors are the primary mode of transmission of some pathogens (e.g., *Streptobacillus monilliformis*, and Seoul hantavirus; Himsworth et al., 2013b, migration events could promote disease spread. Indeed, Lee et al.

TABLE 1 | Conclusions from a review of the published literature describing Norway and black rat (*Rattus norvegicus* and *Rattus rattus*) movement in urban centers.

Knowledge gained from this review	• Urban rat movement is dependent on access to important resources (i.e., food and harborage)
	• Rat movement is impeded by barriers such as roads and waterways
	• Population genetics-based tools demonstrate that rats may cross roads more often than previously estimated using trapping-based techniques
	• Long-distance dispersal events are infrequent but can occur over a distance of several kilometers, facilitating connectivity among distant populations
	• Rat immigration is not always successful, with some migrating individuals being evicted by resident populations
	• Rats may change their home range and natural movement patterns in response to environmental change and anthropogenic habitat modification
Remaining knowledge gaps	• How do specific environmental and socioeconomic features of the urban environment promote or hinder rat movement?
	• Why do rats disperse? Is there a consistent answer or variety of reasons for dispersal?
	• What is the dispersal kernel (i.e., the distribution of dispersal distances) of rats?
	• How does habitat disturbance (e.g., demolition) affect rat movement, and how do proactive approaches to rat control minimize these effects?
	• How do pest control initiatives which are scaled to the level of rat movement (e.g., eradication units) compare to traditional pest control approaches? How can information on rat movement be used to inform innovative pest control technologies?
	• What is the role of rat movement in the transmission of rat-associated pathogens among populations, and how does this affect pathogen prevalence?

(2018) demonstrated that employing lethal control techniques may increase pathogen prevalence among remaining rats. This increase could be due, in part, to the effects of pest control on rat movement, and underscores the potential role of methods which reduce rat population size without prompting migration (e.g., rat birth control, gene drive technologies). Therefore, to monitor and mitigate the potential health risks posed by rats, public health officials require information on the distribution of rat-associated pathogens, the role of movement in the transmission of pathogens among urban rats, and how different approaches to pest control can minimize these risks.

Limitations

Although one of the aims of this review was to compare and contrast data across study locations, deriving quantitative estimates of urban rat movement patterns is difficult due to the limited number of studies evaluating urban rat movement, and an over-representation of research in developed countries (**Supplementary Table 2**). These limitations are compounded by differences between, and limitations of, the included studies. First, rat movement estimates have been derived using various methods which have a suite of limitations (see **Box 1**). These limitations highlight the challenges of studying the movements of not only rats (Parsons et al., 2017; Desvars-Larrive et al., 2018), but urban wildlife in general (LaPoint et al., 2015). Second, even among studies which employ similar techniques to measure movement (i.e., CMR), researchers have used a variety of models for calculating home range size (Low et al., 2013; Oyedele et al., 2015). In tandem, these differences limit the ability to make direct comparisons. Yet, these issues emphasize the importance of employing multiple tools to address methodological limitations (e.g., combining continuous, trapping-, and genetics-based methods) and utilizing either multiple or standardized calculation methods to estimate movement parameters to foster comparability amongst studies.

CONCLUSIONS

Overall, this review highlights a number of important features with regards to rat movement and underscores their significance for stakeholders addressing urban rat infestations. Despite the information synthesized in this review, a number of important questions remain (**Table 1**). To address these knowledge gaps, we suggest that future research prioritize collaborative, multi-jurisdictional research which incorporates multiple methods and standardized approaches to measure rat movement.

DATA AVAILABILITY

All data relevant to this review is included in the manuscript and the supplementary file.

AUTHOR CONTRIBUTIONS

This review was conceived of by KB and designed by KB and CH. Inclusion criteria was determined by KB and ML who also screened and extracted relevant information from all papers. The first draft of the manuscript was written by KB with substantial contributions by ML and CH. DP provided feedback on earlier drafts of the manuscript, and all authors approved the final version for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00013/full#supplementary-material

REFERENCES

Abdelkrim, J., Pascal, M., and Samadi, S. (2007). Establishing causes of eradication failure based on genetics: case study of ship rat eradication in Ste. *Anne Archipelago. Conserv. Biol*. 21, 719–730. doi: 10.1111/j.1523-1739.2007.00696.x

Adams, A. L., van Heezik, Y., Dickinson, K. J. M., and Robertson, B. C. (2014). Identifying eradication units in an invasive mammalian pest species. *Biol. Invasions* 16, 1481–1496. doi: 10.1007/s10530-013-0586-9

Almeida, A., Corrigan, R., and Sarno, R. (2013). The economic impact of commensal rodents on small businesses in Manhattan's chinatown: trends and possible causes. *Sub. Sust*. 1:2. doi: 10.5038/2164-0866.1.1.2

Andrews, R. V., and Belknap, R. W. (1983). Efficacy of alpha-chlorhydrin in sewer rat control. *J. Hyg*. 91, 359–366.

Angley, L. P., Combs, M., Firth, C., Frye, M. J., Lipkin, I., Richardson, J. L., et al. (2018). Spatial variation in the parasite communities and genomic structure of urban rats in New York City. *Zoonoses Public Health* 65, e113–e123. doi: 10.1111/zph.12418

Animal Care Use Committee (1998). Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *J. Mammal*. 79, 1416–1431. doi: 10.2307/1383033

Arai, L., Britten, N., Popay, J., Roberts, H., Petticrew, M., Rodgers, M., et al. (2007). Testing methodological developments in the conduct of narrative synthesis: a demonstration review of research on the implementation of smoke alarm interventions. *Evid. Policy* 3, 361–383. doi: 10.1332/174426407781738029

Barnett, S. A. (1963). *The Rat: A Study in Behaviour*. Chicago, IL: Aldine Publishing Co.

Barnett, S. A., and Bathard, A. H. (1953). Population dynamics of sewer rats. *J. Hyg*. 51, 483–491.

Barnett, S. A., Bathard, A. H., and Spencer, M. M. (1951). Rat populations and control in two English villages. *Ann. Appl. Biol*. 38, 444–463.

Battersby, S. A., Parsons, R., and Webster, J. P. (2002). Urban rat infestations and the risk to public health. *J. Environ. Health Res*. 1, 4–12.

Bentley, E. W., Bathard, A. H., and Riley, J. D. (1958). The control of rats living between access points in sewers. *J. Hyg*. 56, 19–28.

Bentley, E. W., Bathard, A. H., and Riley, J. D. (1959). The rates of recovery of sewer rat populations after poisoning. *J. Hyg*. 57, 291–298.

Berthier, K., Garba, M., Leblois, R., Navascués, M., Tatard, C., Gauthier, P., et al. (2016). Black rat invasion of inland Sahel: insights from interviews and population genetics in south-western Niger. *Biol. J. Linn. Soc*. 119, 748–765. doi: 10.1111/bij.12836

Bomford, M., and O'Brien, P. (1995). Eradication or control for vertebrate pests? *Wildl. Soc. Bull*. 23, 249–255.

Börger, L., Franconi, N., Ferretti, F., Meschi, F., De Michele, G., Gantz, A., et al. (2006). An integrated approach to identify spatiotemporal and individual-level determinants of animal home range size. *Am. Nat*. 168, 471–485. doi: 10.1086/507883

Burt, W. H. (1943). Territoriality and home range concepts as applied to mammals. *J. Mammal*. 24, 346–352.

Byers, K. A., Lee, M. J., Donovan, C. M., Patrick, D. M., and Himsworth, C. G. (2017). A novel method for affixing global positioning system (GPS) tags to urban Norway rats (*Rattus norvegicus*): feasibility, health impacts and potential for tracking movement. *J. Urban Ecol*. 3:e68496. doi: 10.1093/jue/jux010

Cagnacci, F., Boitani, L., Powell, R. A., and Boyce, M. S. (2010). Animal ecology meets GPS-based radiotelemetry: a perfect storm of opportunities and challenges. *Phil. Trans. R. Soc. B*. 365, 2157–2162. doi: 10.1098/rstb.2010.0107

Calhoun, J. B. (1948). Mortality and movement of brown rats (*Rattus norvegicus*) in artificially supersaturated populations. *J. Wildl. Manage*. 12, 167–172.

Calhoun, J. B. (1963). *The Ecology and Sociology of the Norway Rat*. Bethesda, MD: US Department of health, Education and Welfare.

Cederlund, G., and Sand, H. (1994). Home-range size in relation to age and sex in moose. *J. Mammal*. 75, 1005–1012.

Childs, J. E., McLafferty, S. L., Sadek, R., Miller, G. L., Khan, A. S., DuPree, E. R., et al. (1998). Epidemiology of rodent bites and prediction of rat infestation in New York city. *Am. J. Epidemiol*. 148, 78–87.

Chitrakar, R. M., Baker, D. C., and Guaralda, M., (2016). Urban growth and development of contemporary neighbourhood public space in Kathmandu Valley, Nepal. *Habitat Int*. 53, 30–38. doi: 10.1016/j.habitatint.2015.11.006

Colvin, B. A., and Jackson, W. B. (1999). "Urban rodent control programs for the 21st century," in *Ecologically-Based Rodent Management*, eds G. R. Singleton, H. Liers, and Z. Zhang (Canberra, ACT: Australian Centre for International Agricultural Research), 243–257.

Colvin, B. A., Swift, T. B., and Fothergill, F. E. (1998). "Control of Norway rats in sewer and utility systems using pulsed baiting methods," in *Proceedings of the Eighteenth Vertebrate Pest Conference* (Davis, CA), 247–253.

Combs, M., Byers, K. A., Ghersi, B. M., Blum, M. J., Caccone, A., Costa, F., et al. (2018a). Urban rat races: spatial population genomics of brown rats (*Rattus norvegicus*) compared across multiple cities. *Proc. R. Soc. B*. 285:20180245. doi: 10.1098/rspb.2018.0245

Combs, M., Puckett, E. E., Richardson, J., Mims, D., and Munshi-South, J. (2018b). Spatial population genomics of the brown rat (*Rattus norvegicus*) in New York City. *Mol. Ecol*. 27, 83–98. doi: 10.1111/mec.14437

Cooke, S. J., Hinch, S. G., Wikelski, M., Andrews, R. D., Kuchel, L. J., Wolcott, T. G., et al. (2004). Biotelemetry: a mechanistic approach to ecology. *Trends Ecol. Evol*. 19, 334–343. doi: 10.1016/j.tree.2004.04.003

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., et al. (2015a). Global morbidity and mortality of leptospirosis: a systematic review. *PLoS Negl. Trop. Dis*. 9:e0003898.doi: 10.1371/journal.pntd.0003898

Costa, F., Richardson, J. L., Dion, K., Mariani, C., Pertile, A. C., Burak, M. K., et al. (2016). Multiple paternity in the Norway rat, *Rattus norvegicus*, from urban slums in Salvador, Brazil. *J. Hered*. 107, 181–186. doi: 10.1093/jhered/esv098

Costa, F., Wunder, E. A. Jr., De Oliveira, D., Bisht, V., Rodrigues, G., Reis, M. G., et al. (2015b). Patterns in *Leptospira* shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission. *PLoS Negl. Trop. Dis*. 9:e0003819. doi: 10.1371/journal.pntd.0003819

Creel, R. H. (1915). The migratory habits of rats: with special reference to the spread of plague. *Public Health Rep*. 30, 1679–1685.

Dahle, B., and Swenson, J. E. (2003). Home ranges in adult Scandinavian brown bears (*Ursus arctos*): effect of mass, sex, reproductive category, population density and habitat type. *J. Zool*. 260, 329–335. doi: 10.1017/S0952836903003753

Davis, D. E., and Christian, J. J. (1956). Changes in Norway rat populations induced by introduction of rats. *J. Wildl. Manage* 20, 378–383.

Davis, D. E., Emlen, J. T., and Stokes, A. W. (1948). Studies on home range in the brown rat. *J. Mammal*. 29, 207–225.

Desvars-Larrive, A., Baldi, M., Walter, T., Zink, R., and Walzer, C. (2018). Brown rats (*Rattus norvegicus*) in urban ecosystems: are the constraints related to fieldwork a limit to their study? *Urban Ecosyst*. 21, 951–964. doi: 10.1007/s11252-018-0772-8

Desvars-Larrive, A., Pascal, M., Gasqui, P., Cosson, J.-F., Benoît, E., Lattard, V., et al. (2017). Population genetics, community of parasites, and resistance to rodenticides in an urban brown rat (*Rattus norvegicus*) population. *PLoS ONE* 12:e0184015. doi: 10.1371/journal.pone.0184015

Dickman, C. R., and Doncaster, C. P. (1987). The ecology of small mammals in urban habitats. 1. Populations in a patchy environment. *J. Anim. Ecol*. 56, 629–640. doi: 10.2307/5073

Dowding, J. E., and Murphy, E. C. (1994). Ecology of ship rats (*Rattus rattus*) in a kauri (*Agathis australis*) forest in Northland, New Zealand. *NZ. J. Ecol.* 18, 19–28. doi: 10.2307/24053652

Downs, J. A., and Horner, M. W. (2008). Effects of point pattern shape on home-range estimates. *J. Wildl. Manage.* 72, 1813–1818. doi: 10.2193/2007-454

Drickamer, L. C. (1987). Behavioral aspects of rodent population biology. *Am. Zool.* 27, 953–969.

Emlen, J. T., Stokes, A. W., and Davis, D. E. (1949). Methods for estimating populations of brown rats in urban habitats. *Ecology* 30, 430–442.

Feng, A. Y. T., and Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosyst.* 17, 149–162. doi: 10.1007/s11252-013-0305-4

Gardner-Santana, L. C., Norris, D. E., Fornadel, C. M., Hinson, E. R., Klein, S. L., and Glass, G. E. (2009). Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*). *Mol. Ecol.* 18, 2766–2778. doi: 10.1111/j.1365-294X.2009.04232.x

Garrard, J. (2013). *Health Sciences Literature Review Made Easy*. Burlington, MA: Jones and Bartlett Learning.

German, D., and Latkin, C. A. (2016). Exposure to urban rats as a community stressor among low-income urban residents. *J. Community Psychol.* 44, 249–262. doi: 10.1002/jcop.21762

Glass, G. E., Childs, J. E., Korch, G. W., and Leduc, J. W. (1989). Comparative ecology and social interactions of Norway rat (*Rattus norvegicus*) populations in Baltimore, Maryland. *Occas. Pap. Mus. Nat. Hist.* 130, 1–33.

Glass, G. E., Klein, S. L., Norris, D. E., and Gardner, L. C. (2016). Multiple paternity in urban Norway rats: extended ranging for mates. *Vector Borne Zoo. Dis.* 16, 342–348. doi: 10.1089/vbz.2015.1816

Gras, L. M., Patergnani, M., and Farina, M. (2012). Poison-based commensal rodent control strategies in urban ecosystems: some evidence against sewer-baiting. *EcoHealth* 9, 75–79. doi: 10.1007/s10393-012-0748-8

Greaves, J. H., Hammond, L. E., and Bathard, A. H. (1968). The control of re-invasion by rats of part of a sewer network. *Ann. Appl. Biol.* 62, 341–351.

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., et al. (2008). Global change and the ecology of cities. *Science* 319, 756–760. doi: 10.1126/science.1150195

Guivier, E., Galan, M., Chaval, Y., Xuéreb, A., Ribas Salvador, A., Poulle, M. L., et al. (2011). Landscape genetics highlights the role of bank vole metapopulation dynamics in the epidemiology of Puumala hantavirus. *Mol. Ecol.* 20, 3569–3583. doi: 10.1111/j.1365-294X.2011.05199.x

Hacker, K. P., Minter, A., Begon, M., Diggle, P. J., Serrano, S., Reis, M. G., et al. (2016). A comparative assessment of track plates to quantify fine scale variations in the relative abundance of Norway rats in urban slums. *Urban Ecosyst.* 19, 561–575. doi: 10.1007/s11252-015-0519-8

Hayne, D. W. (1949). Calculation of size of home range. *Am. Soc. Mammal.* 30, 1–18.

Heiberg, A.-C., Sluydts, V., and Leirs, H. (2012). Uncovering the secret lives of sewer rats (*Rattus norvegicus*): movements, distribution and population dynamics revealed by a capture-mark-recapture study. *Wildl. Res.* 39, 202–219. doi: 10.1071/WR11149

Himsworth, C. G., Bai, Y., Kosoy, M. Y., Wood, H., DiBernardo, A., Lindsay, R., et al. (2015). An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul Hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: is pathogen presence a reflection of global and local rat population structure? *Vector Borne Zoo. Dis.* 15, 21–26. doi: 10.1089/vbz.2014.1657

Himsworth, C. G., Bidulka, J., Parsons, K. L., Feng, A. Y. T., Tang, P., Jardine, C. M., et al. (2013a). Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. *PLoS Negl. Trop. Dis.* 7:e2270. doi: 10.1371/journal.pntd.0002270.s001

Himsworth, C. G., Parsons, K. L., Feng, A. Y. T., Kerr, T., Jardine, C. M., and Patrick, D. M. (2014a). A mixed methods approach to exploring the relationship between Norway rat (*Rattus norvegicus*) abundance and features of the urban environment in an inner-city neighborhood of Vancouver, Canada. *PLoS ONE* 9:e97776. doi: 10.1371/journal.pone.0097776.s004

Himsworth, C. G., Parsons, K. L., Jardine, C., and Patrick, D. M. (2013b). Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne and Zoo. Dis.* 13, 349–359. doi: 10.1089/vbz.2012.1195

Himsworth, C. G., Patrick, D. M., Mak, S., Jardine, C. M., Tang, P., and Weese, J. S. (2014b). Carriage of *Clostridium difficile* by wild urban Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*). *Appl. Environ. Microbiol.* 80, 1299–1305. doi: 10.1128/AEM.03609-13

Kajdacsi, B., Costa, F., Hyseni, C., Porter, F., Brown, J., Rodrigues, G., et al. (2013). Urban population genetics of slum-dwelling rats (*Rattus norvegicus*) in Salvador, Brazil. *Mol. Ecol.* 22, 5056–5070. doi: 10.1111/mec.12455

King, O. M. (1950). An ecological study of the Norway rat and the house mouse in a city block in Lawrence, Kansas. *Trans. Kansas Acad. Sci.* 53, 500–528. doi: 10.2307/3625896

Kjellander, P., Hewison, A. J. M., Liberg, O., Angibault, J. M., Bideau, E., and Cargnelutti, B. (2004). Experimental evidence for density-dependence of home-range size in roe deer (*Capreolus capreolus* L.): a comparison of two long-term studies. *Oecologia* 139, 478–485. doi: 10.1007/s00442-004-1529-z

Lajeunesse, M. J. (2016). Facilitating systematic reviews, data extraction and meta-analysis with the METAGEAR package for R. *Methods Ecol. Evol.* 7, 323–330. doi: 10.1111/2041-210X.12472

Lam, R., Byers, K. A., and Himsworth, C. G. (2018). Beyond zoonosis: the mental health impacts of rat exposure on impoverished urban neighborhoods. *J. Environ. Health.* 81, 8–12.

LaPoint, S., Balkenhol, N., Hale, J., Sadler, J., and van der Ree, R. (2015). Ecological connectivity research in urban areas. *Funct. Ecol.* 29, 868–878. doi: 10.1111/1365-2435.12489

Lee, M. J., Byers, K. A., Donovan, C. M., Bidulka, J. J., Stephen, C., Patrick, D. M., et al. (2018). Effects of culling on *Leptospira interrogans* carriage by rats. *Emerg. Infect. Dis.* 24, 356–360. doi: 10.3201/eid2402.171371

Low, B. W., Mills, H., Algar, D., and Hamilton, N. (2013). Home ranges of introduced rats on Christmas Island: a pilot study. *Ecol. Manag. Restor.* 14, 41–46. doi: 10.1111/emr.12024

Mangombi, J. B., Brouat, C., Loiseau, A., Banga, O., Leroy, E. M., Bourgarel, M., et al. (2016). Urban population genetics of the invasive black rats in Franceville, Gabon. *J. Zool.* 299, 183–190. doi: 10.1111/jzo.12334

Moher, D., Liberati, A., Tetzlaff, J., Altzman, D. G., and The PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. *PLoS Med.* 6:e1000097. doi: 10.1371/journal.pmed.1000097

Moro, D., Byrne, M., Kennedy, M., Campbell, S., and Tizard, M. (2018). Identifying knowledge gaps for gene drive research to control invasive animal species: the next CRISPR step. *Global Ecol. Conserv.* 13:e00363. doi: 10.1016/j.gecco.2017.e00363

Oyedele, D. T., Sah, S. A. M., Kairuddin, L., Mohd, W., and Ibrahim, M. W. (2015). Range measurement and a habitat suitability map for the Norway rat in a highly developed urban environment. *Trop. Life Sci. Res.* 26, 27–44.

Parsons, M. B., Gillespie, T. R., Lonsdorf, E. V., Travis, D., Lipende, I., Gilagiza, B., et al. (2014). Global positioning system data-loggers: a tool to quantify fine-scale movement of domestic animals to evaluate potential for zoonotic transmission to an endangered wildlife population. *PLoS ONE* 9:110984. doi: 10.1371/journal.pone.0110984

Parsons, M. H., Banks, P. B., Deutsch, M. A., Corrigan, R. F., and Munshi-South, J. (2017). Trends in urban rat ecology: a framework to define the prevailing knowledge gaps and incentives for academia, pest management professionals (PMPs) and public health agencies to participate. *J. Urban Ecol.* 3, 1–8. doi: 10.1093/jue/jux005

Parsons, M. H., Sarno, R. J., and Deutsch, M. A. (2015). Jump-starting urban rat research: conspecific pheromones recruit wild rats into a behavioral and pathogen-monitoring assay. *Front. Ecol. Evol.* 3:146. doi: 10.3389/fevo.2015.00146

Pawe, C. K., and Saikia, A. (2018). Unplanned urban growth: land use/land cover change in the Guwahati Metropolitan Area, India. Geogr. *Tidsskr Den.* 118, 88–100. doi: 10.1080/00167223.2017.1405357

Petrie, G. F., and Todd, R. E. (1923). Observations in Upper Egypt on the range of excursion of the house rodents: *R. rattus* and *Acomys cahirinus*. *Rep. Notes Pub. Hlth. Lab. Cairo* 5, 14–18.

Petrie, G. F., Todd, R. E., Skander, R., and Hilmy, F. (1924). A report on plague investigations in Egypt. *J. Hyg.* 23, 117–150.

Pimentel, D., Zuniga, R., and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52, 273–288. doi: 10.1016/j.ecolecon.2004.10.002

Quixabeira-Santos, J. C., Medaglia, M. L. G., Pescador, C. A., and Damaso, C. R. (2011). Animal movement and establishment of vaccinia virus cantagalo strain in amazon biome, Brazil. *Emerg. Infect. Dis*. 17, 726–729. doi: 10.3201/eid1704.101581

Recht, M. A. (1982). The fine structure of the home range and activity pattern of free-ranging telemetered urban Norway rats, *Rattus norvegicus* (Berkenhout). *Bull. Soc. Vector Ecol*. 7, 29–36.

Recht, M. A., Geck, R., and Challet, G. L. (1983). The fine-structure of the home range and activity-phasing of unrestricted telemetered urban roof rats, *Rattus rattus* in Orange County, California. *Bull. Soc. Vector Ecol*. 8, 51–64.

Richardson, J. L., Burak, M. K., Hernandez, C., Shirvell, J. M., Mariani, C., Carvalho-Pereira, T. S. A., et al. (2017). Using fine-scale spatial genetics of Norway rats to improve control efforts and reduce Leptospirosis risk in urban slum environments. *Evol. Appl*. 10, 323–337. doi: 10.1111/eva.12449

Richter, C. P. (1968). Experiences of a reluctant rat-catcher the common Norway rat - friend or enemy? *Proc. Amer. Phil. Soc*. 112, 403–415.

Robertson, B. C., and Gemmell, N. J. (2004). Defining eradication units to control invasive pests. *J. Appl. Ecol*. 41, 1042–1048. doi: 10.1111/j.0021-8901.2004.00984.x

Rondinini, C., and Doncaster, C. P. (2002). Roads as barriers to movement for hedgehogs. *Funct. Ecol*. 16, 504–509. doi: 10.1046/j.1365-2435.2002.00651.x

Safi, K., König, B., and Kerth, G. (2007). Sex differences in population genetics, home range size and habitat use of the parti-colored bat (*Vespertilio murinus*, Linnaeus 1758) in Switzerland and their consequences for conservation. *Biol. Cons*. 137, 28–36. doi: 10.1016/j.biocon.2007.01.011

Takahashi, L. K., and Lore, R. K. (1980). Foraging and food hoarding of wild *Rattus norvegicus* in an urban environment. *Behav. Neural Biol*. 29, 527–531.

Tanaka, R. (1963). On the problem of trap-response types of small mammal populations. *Res. Popul. Ecol*. 5, 139–146.

Tanaka, R., and Kawashima, F. (1951). Movement and population of the brown rat in Kochi City. *Annot. Zool. Jpn*. 24, 225–233.

Taylor, K. D. (1978). Range of movement and activity of common rats (*Rattus norvegicus*) on agricultural land. *J. Appl. Ecol*. 15, 663–677. doi: 10.2307/2402767

Tomkiewicz, S. M., Fuller, M. R., Kie, J. G., and Bates, K. K. (2010). Global positioning system and associated technologies in animal behaviour and ecological research. *Phil. Trans. R. Soc. B*. 365, 2163–2176. doi: 10.1098/rstb.2010.0090

Traweger, D., and Slotta-Bachmayr, L. (2005). Introducing GIS-modelling into the management of a brown rat (*Rattus norvegicus* Berk.) (Mamm. *Rodentia Muridae)* population in an urban habitat. *J. Pest Sci*. 78, 17–24. doi: 10.1007/s10340-004-0062-5

Tucker, M. A., Böhning-Gaese, K., Fagan, W. F., Fryxell, J. M., Van Moorter, B., Alberts, S. C., et al. (2018). Moving in the Anthropocene: global reductions in terrestrial mammalian movements. *Science* 359, 466–469. doi: 10.1126/science.aam9712

United Nations (2018). *World Urbanization Prospects: The 2018 Revision*. New York, NY: United Nations.

Volkova, V. V., Howey, R., Savill, N. J., and Woolhouse, M. E. J. (2010). Sheep movement networks and the transmission of infectious diseases. *PLoS ONE* 5:e11185. doi: 10.1371/journal.pone.0011185

Wiktander, U., Olsson, O., and Nilsson, S. G. (2001). Seasonal variation in home-range size, and habitat area requirement of the lesser spotted woodpecker (*Dendrocopos minor*) in southern Sweden. *Biol. Cons*. 100, 387–395. doi: 10.1016/S0006-3207(01)00045-3

Wolff, J. O. (1985). The effects of density, food, and interspecific interference on home range size in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Can. J. Zool*. 63, 2657–2662.

Worth, C. B. (1950). Field and laboratory observations on roof rats, *Rattus rattus* (Linnaeus), in Florida. *J. Mammal*. 31, 293–304.

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Tails of Two Cities: Age and Wounding Are Associated With Carriage of *Leptospira interrogans* by Norway Rats (*Rattus norvegicus*) in Ecologically Distinct Urban Environments

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Leptospirosis is a zoonotic disease for which rats are the primary reservoir in urban environments. It is transmitted from rats to people via urine, and is responsible for significant human morbidity and mortality in under-resourced settings. To mitigate the risks posed to people, it is important to understand the ecology of the causative agent *Leptospira interrogans*. The overarching objective of this study was to compare *L. interrogans* carriage in urban Norway rats in two ecologically distinct urban environments. We trapped Norway rats (*Rattus norvegicus*) in Vancouver, Canada ($N = 525$) and Salvador, Brazil ($N = 433$) to evaluate whether rat characteristics (i.e., sex, weight, sexual maturity, pregnancy, and the presence of wounds) and location of capture were associated with *L. interrogans* status. Using generalized linear mixed models to control for clustering by trapping location, we found a greater prevalence of *L. interrogans* in Salvador (79%) than in Vancouver (12%), and greater spatial heterogeneity in pathogen prevalence in Vancouver than in Salvador. In both locations, we found that older rats and rats with more bite wounds had greater odds of *L. interrogans* carriage, although wounding influenced pathogen status more for younger animals. Additionally, we found that juvenile rats in Salvador were more likely to leave the nest infected with *L. interrogans* than were rats in Vancouver, suggesting that potential differences in early-life transmission dynamics exist between the two locations. Together, these results elucidate both general *L. interrogans* ecology, as well as the importance of geographical location in determining transmission among rats.

Keywords: age-prevalence, bite wound, intraspecific transmission leptospira, leptospirosis, Norway rat (*Rattus norvegicus*), urban ecology

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INTRODUCTION

Leptospirosis is a zoonotic disease with a global distribution that is responsible for significant human morbidity and mortality (Himsworth et al., 2013b; Costa et al., 2015a). Residents of resource-poor countries are particularly affected (Costa et al., 2015a). It is caused by the spirochete bacterium *Leptospira interrogans*, and symptoms can range from undifferentiated febrile illness to fatal liver failure or pulmonary hemorrhage. Rats, particularly Norway rats (*Rattus norvegicus*), are the primary reservoir for *L. interrogans* in urban centers (Costa et al., 2014b). The bacterium is carried asymptomatically in the rat kidneys and shed in the urine (Costa et al., 2015b). It is transmitted from rats to humans primarily via direct or indirect contact with rat urine in the urban environment (Evangelista and Coburn, 2010). The incidence and distribution of urban leptospirosis are increasing in association with global urbanization (Hartskeerl et al., 2011), making it an important and growing public health concern.

An understanding of the ecology of *L. interrogans* in urban rats is critical for monitoring and mitigating human health risks. Past work has shown that carriage of *L. interrogans* by rats is associated with a variety of factors, including age, sex, sexual maturity, weight, internal fat volume, the presence and severity of bite wounds, and location of capture (Thiermann, 1977; Easterbrook et al., 2007; Krøjgaard et al., 2009; Himsworth et al., 2013a; Costa et al., 2014a, 2015b; Minter et al., 2017; Lee et al., 2018). However, results have varied across studies. For example, Minter et al. (2017) documented evidence of juvenile rats leaving the nest with infection, which could reflect vertical transmission of *L. interrogans* from mothers to pups (De Oliveira et al., 2016), while others have suggested that transmission occurs almost exclusively among adults (Himsworth et al., 2013a). Additionally, the overall prevalence of *L. interrogans* carriage has varied markedly among studies (Aviat et al., 2009; Koizumi et al., 2009; Scialfa et al., 2010; Maas et al., 2018). Unfortunately, it is difficult to determine if past findings reflect true variations in the ecology of *L. interrogans* in different cities, or if it is simply a result of differing study methodologies or areas of focus. For the same reason, the driving forces behind results shared among studies are hard to elucidate. For example, regardless of the city, variation in *L. interrogans* prevalence by trapping site has been consistently observed (Krøjgaard et al., 2009; Himsworth et al., 2013a; Costa et al., 2015b; Lee et al., 2018); however, differences in both sampling approach and statistical analyses among studies have made it difficult to determine the relative impact of geographical location on the constellation of factors affecting *L. interrogans* ecology.

Indeed, there has yet to be a study directly comparing *L. interrogans* carriage between two significantly different urban environments. These sorts of comparisons are essential for determining which elements of *L. interrogans* ecology are unique to a location, and which can be extrapolated among urban environments, as well as for clarifying the overall ecology of the pathogen. The aim of this study was to compare and contrast the characteristics of *L. interrogans* carriage in urban Norway rats in Salvador, Brazil, and Vancouver, Canada. This was accomplished through collation and re-analysis of data collected by two methodologically aligned studies (Himsworth et al., 2013a; Minter et al., 2017).

Abbreviations: DTES, Downtown Eastside.

METHODS

Ethics Statement

This study was approved by the University of British Columbia's Animal Care Committee (A11-0087) and adhered to national guidelines set out by the Canadian Council on Animal Care. Protocols of this study were also approved by the Institutional Animal Care and Use Committee (IACUC) from Brazil and United States. At Oswaldo Cruz Foundation, Salvador, Brazil, the Comissão de Ética no Uso de Animais (CEUA) do CPqGM-FIOCRUZ-BA approved the protocol number 003/2012.

Study Sites

In Vancouver, the study area was comprised of 43 contiguous city blocks encompassing the core of Vancouver's Downtown Eastside (DTES) (N49°17′ / W123°6′). Also included was an area within the port terminal, which is a center for international shipping that forms the northern border of the DTES (see **Figure 1**). The DTES is an inner-city area of low socioeconomic status known as "Canada's poorest postal code". While the built environment is highly developed, with densely populated city blocks (e.g., including residential, commercial, and industrial parcels) surrounded by roadways, buildings are antiquated and often in disrepair. Additionally, there is considerable refuse accumulated in the alleyways, which are also heavily used by residents for injecting drugs, sleeping, loitering, etc (Smith, 2000). Vancouver has a moderate oceanic climate with an average annual temperature of 11°C and average annual precipitation of 1,588 mm.

In Salvador, the study area included three valleys within Pau da Lima (S13°32′ / W38°43′), an urban slum. This urban area is characterized by a lack of basic sanitation, open and free flowing sewers as well as poor housing conditions (Felzemburgh et al., 2014) (**Figure 1**). Salvador has a subtropical climate with an average annual temperature of 25°C and average annual precipitation of 1,781 mm.

Trapping

In Vancouver, each block (and the port site) was randomly assigned to a 3-week study period over the course of 1 year (September 2011–August 2012). Within each block, ∼20 Tomahawk Rigid Traps for rats (Tomohawk Live Trap, Hazlelhurst, USA), which trap rats alive, were set out along each side of the back alley that bisected the block. At the port, traps were placed in areas where port staff had observed rats. Traps were pre-baited (filled with bait but fixed open) for 1 week, followed by 2 weeks of active trapping. Baits used included a combination of peanut butter, bacon fat, and oats. Traps were set in the evening and trapped rats were collected the following morning.

In Salvador, 150 sampling points were randomly selected and a 30 m^2 buffer zone was placed around each sampling point.

FIGURE 1 | Trapping locations in the 43 city blocks of Vancouver, Canada **(A)** and three valleys of Salvador, Brazil **(B)**. Images provided through Google Earth Professional (https://www.google.com/earth/download/gep/agree.html). Examples of areas where rats were caught in each location are indicated in **(C)** (Vancouver), **(D,E)** (Salvador).

Three trapping sites were selected within each sampling point based on signs of rat presence, and two Tomahawk traps were placed at each sampling point. Trapping occurred during four periods of time (May–August 2013, October–December 2013, March–August 2014, September–December 2014). Traps were pre-baited for 2 to 3 days, followed by 4 to 6 consecutive days of active trapping. Traps were baited with sausage. Traps were set at sundown and trapped rats were collected at sunrise the following day.

Data Collection

In Vancouver, trapped rats were anesthetized by isoflurane inhalation prior to pentobarbital euthanasia via intracardiac injection. Rats underwent a complete necropsy during which kidney samples were collected and stored at −80°C. In Salvador, rats were anesthetized by isoflurane inhalation prior to thiopental euthanasia via intraperitoneal injection. Urine was obtained directly from the bladder using a 1 mL syringe and stored at −80°C.

For both cities, the following demographic and morphometric data were collected: species (based on external morphology), sex, weight, sexual maturity (scrotal testes for males and perforate vagina for females), pregnancy, and the presence of bite wounds. The date and location of each rat trapped was also recorded.

The Maxwell 16 System DNA Purification Kit (Promega Corp., USA) and the QiaAMP DNA Mini Kit (Qiagen Inc., Canada), was used to extract DNA from the urine and kidney samples, respectively. DNA extracts were then analyzed using a real-time PCR assay which targets a 242 bp fragment of the *lipL32* gene of pathogenic *Leptospira* species (Stoddard et al., 2009). It should be noted that a near perfect correlation between leptospiral loads in the urine and kidneys of wild, urban Norway rats has been identified, suggesting that urine and kidney samples can likely be used interchangeably for the study of *L. interrogans* in this species (Costa et al., 2015b).

Statistical Analysis

Previous studies have indicated that age has a strong, positive association with *L. interrogans* carriage in urban rats (Krøjgaard et al., 2009; Himsworth et al., 2013a; Costa et al., 2015b; Minter et al., 2017). However, the majority of these studies used weight as a proxy for age, which is problematic since weight and age are not

linearly associated with each other (Calhoun, 1963). In order to overcome this issue, the von Bertalanffy equation (weight = a[1 – exp{–r(age – c)}], where "a" is the asymptote, "r" is the constant growth rate and "c" is the age at which maximum growth occurs) has been used to more accurately model rodent age curves, including those of urban rats (Burthe et al., 2010; Minter et al., 2017). For this study rats were aged using the von Bertalanffy equation using parameters from Calhoun (1963) as in Minter et al. (2017). In pregnant females, weight was adjusted by the average weight difference between pregnant and non-pregnant, sexually mature females prior to applying the age calculation (Minter et al., 2017).

Explanatory variables included age (in days), sexual maturity (yes/no), sex (male/female), and bite wounds (absent/present) as these variables were previously identified as risk factors for *L. interrogans* carriage in Vancouver and/or Salvador (Himsworth et al., 2013a; Minter et al., 2017). Chi-squared test or Welch's *t*-test were used to compare the distribution of the variables between Vancouver and Salvador. Linear regression was used to identify the relationship between age and wounding. Neither weight nor body length (another proxy for age in rats) were included, as these variables were strongly collinear with age, which was the main variable under consideration.

Multivariate generalized linear mixed models (GLMMs) were used to compare the relationships among *L. interrogans* carriage (positive/negative on PCR) and the aforementioned explanatory variables in Vancouver and Salvador controlling for clustering by trapping location (city block in Vancouver and sampling point in Salvador). These included full models, which contained all explanatory variables (as well as an interaction term between age and wounding), and "final" models obtained through backward selection. For final model selection, non-significant explanatory variables were removed from the full model until all variables were statistically significant at a 5% level. All statistics were conducted in R (R Core Team, 2018) using the lme4 package for GLMMs (Bates et al., 2015).

The full GLMM was used to investigate the risk of rats leaving the nest with infection. Specifically, the probability of infection was calculated for the youngest animals in each cohort (27 days for Salvador and 26 days for Vancouver) that had no wounds and were sexually immature.

RESULTS

The final sample included 525 and 433 Norway rats in Vancouver and Salvador, respectively. In both cities there was approximately an equal sex ratio (see **Table 1**). However, the Salvador rats were significantly older (based on the von Bertalanffy estimate for age) and more likely to be sexually mature and wounded compared to the Vancouver rats. In both Vancouver and Salvador, there was a significant positive relationship between wounding and both age and sexual maturity ($p < 0.001$).

The prevalence of *L. interrogans* carriage was higher in Salvador vs. Vancouver (79% vs. 12%). This difference was consistent even after stratifying by age (**Figure 2**). In both the Vancouver and Salvador full models, the odds of *L. interrogans* carriage increased with age and the presence of bite wounds (see **Table 2**) and there was a significant negative interaction between age and bite wounds (indicating that the relative effect of bite wounds was less for older vs. younger rats). The "final" model for both Vancouver and Salvador also included the aforementioned variables. Sex was not significantly related to *L. interrogans* carriage in either city. However, sexual maturity was significant in both the full and final models for Salvador but not for Vancouver. Conversely, the random effect of trapping location on the odd of *L. interrogans* carriage was much greater for Vancouver compared to Salvador (**Table 3**).

TABLE 1 | Baseline characteristics and associations with *Leptospira interrogans* PCR status among Norway rats.

Category	Subcategory	Salvador	Vancouver	*p*-value[a]
		Total (%) ($n = 433$)	Total (%) ($n = 525$)	
Sex	Male	253 (59)	281 (54)	0.1239
	Female	178 (41)	244 (46)	
Sexual maturity	Mature	347 (81)	328 (62)	$p < 0.001$
	Immature	84 (19)	197 (38)	
Age	Median (IQR)	83 (43)	47 (38)	$p < 0.001$
Wound presence	No	192 (45)	386 (74)	$p < 0.001$
	Yes	239 (55)	139 (26)	

[a] *Determined using the Chi-squared test, or Welch's t-test, where appropriate.*

Rats are more likely to be infected with *L. interrogans* at a younger age in Salvador compared to Vancouver (**Figure 2**). The probability of a rat leaving the nest with infection was 0.002 (95% CI: 0–0.01) in Vancouver and 0.21 (95% CI: 0.10–0.32) in Salvador.

DISCUSSION

These results reveal a number of similarities and important differences with regard to the ecology of *L. interrogans* and rats in Vancouver and Salvador. First, it is interesting to note that the rat population in Salvador appeared to be significantly older than that in Vancouver and had a greater proportion of sexually mature rats. The Salvador rats were also more likely to be wounded; however, this may simply be a reflection of population demographics, as older rats and sexually mature rats were more likely to be wounded in both cities. Overall, these results suggest a more rapid population turnover is occurring in Vancouver. Although the reason behind this is unclear, there are several potential explanations. First, rats in Vancouver and Salvador are distinct from each other phylogenetically, and likely arose through independent founder events (Puckett et al., 2016); therefore, there may be heritable differences in lifespan between the two populations. Second, a previous study in Vancouver demonstrated a high prevalence of infectious and degenerative cardiovascular and respiratory disease, particularly in older rats (Rothenburger et al., 2015a,b). While the health status of the

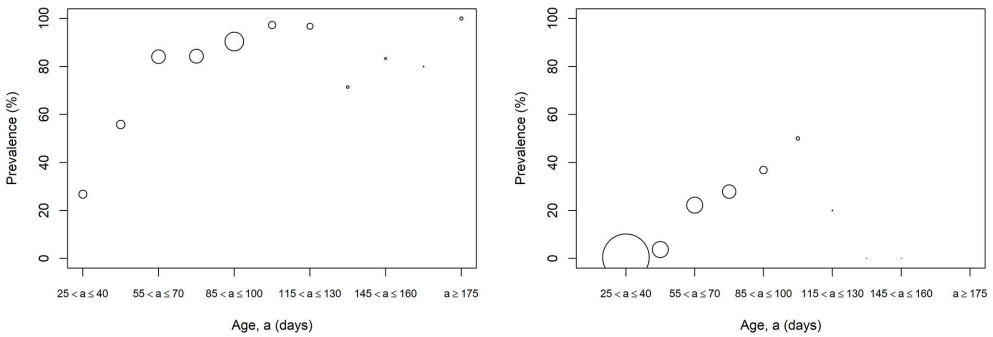

FIGURE 2 | Age-prevalence curve for *Leptospira* infection in Salvador and Vancouver Norway rats (*Rattus norvegicus*). The size of the point indicates the number of rats in that age category (larger point = larger sample size).

TABLE 2 | Odds ratios for being *Leptospira interrogans* PCR-positive among Norway rats.

		Salvador				Vancouver			
		Full model		Final model		Full model		Final model	
Category	**Subcategory**	**OR[a]**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Age		**1.04**	**(1.02, 1.07)**	**1.05**	**(1.02, 1.07)**	**1.09**	**(1.05, 1.13)**	**1.08**	**(1.05, 1.12)**
Sex	Male	0.60	(0.3, 1.19)	–	–	0.53	(0.24, 1.19)	–	–
Maturity	Mature	**3.99**	**(1.72, 9.26)**	**3.19**	**(1.48, 6.89)**	2.06	(0.21, 20.10)	–	–
Wound presence	Yes	**12.29**	**(3.45, 43.75)**	**12.91**	**(3.70, 45.05)**	**21.57**	**(2.92, 159.38)**	**19.43**	**(2.98, 126.87)**
Age*wound presence		**0.96**	**(0.94, 0.99)**	**0.96**	**(0.94, 0.98)**	**0.95**	**(0.91, 0.99)**	**0.95**	**(0.91, 0.98)**

[a]Adjusted by 27 days for Salvador data and by 26 days for Vancouver data. Bold indicates statistical significance at $p < 0.05$.

TABLE 3 | Variance and standard deviation of the random effects (of the intercept) for the Vancouver and Salvador full models.

Random effect	Variance	Standard deviation
Valley (Salvador)	0.898	0.948
Block (Vancouver)	4.35	2.09

Salvador rats is not known, a decreased disease burden in that population compared to Vancouver could account for a longer lifespan. Rats in Salvador and Vancouver exist in two highly disparate environments, and it may be the case that decreased infrastructure and sanitation in Salvador provides greater access to resources (Santos et al., 2017) and supports a longer lifespan in resident rats. Finally, although there are no coordinated rat-control programs in either city, many property and business owners in the DTES conduct lethal rat control (i.e., trapping and poisoning) on their own or through a pest control company, which may contribute to increased population turn over.

Given that *L. interrogans* carriage is strongly associated with age, the higher overall prevalence of the pathogen in Salvador rats may be partially attributable to the different age structures of the two populations. However, after stratifying by age, rats in Vancouver consistently had a lower prevalence of infection compared to those in Salvador. This phenomenon may be caused by the widespread environmental contamination of soil and water with pathogenic *L. interrogans* within the urban slums of Salvador (Casanovas-Massana et al., 2018)–contamination that may be facilitated by the higher average annual temperature and precipitation compared to Vancouver. Indeed, transmission of *L. interrogans* from rats to humans is more common in warmer and wetter climates (Himsworth et al., 2013b). In fact, some studies have suggested that prevalence of *L. interrogans* in rats decreases with increasing latitude simply as a result of climactic differences (Jensen and Magnussen, 2016). Additionally, differences in the built environment between the Salvador and Vancouver study areas make the former more prone to flooding and accumulation of standing water, both of which facilitate environmental transmission of *L. interrogans* (Himsworth et al., 2013b; Hagan et al., 2016). Indeed, it is these differences that likely account for the fact that human cases of leptospirosis are common in Salvador, but have yet to be identified in Vancouver (Costa et al., 2017; McVea et al., 2018).

In both Salvador and Vancouver, the odds of *L. interrogans* infection increased with age. This is a consistent finding among studies of *L. interrogans* in rats (Vanasco et al., 2003; Johnson et al., 2004; Krøjgaard et al., 2009) and likely reflects an increased cumulative probability of exposure to and infection with this pathogen over time. However, it is interesting to note that

rats were significantly more likely to leave the nest carrying *Leptospira* in Salvador compared to Vancouver. Previous studies have identified the presence of *L. interrogans* in breastmilk in Salvador rats (De Oliveira et al., 2016), suggesting the possibility of vertical infection. A similar study has not been conducted in Vancouver; however, it seems unlikely that the core biology of the same species in two separate locations would differ sufficiently to account for significantly different rates of vertical transmission. Additionally, the low number of leptospires in breast milk and tissues may not be sufficient for effective transmission of the bacteria (De Oliveira et al., 2016). It therefore seems more likely that this finding reflects a greater degree of environmental contamination and transmission within the nest in Salvador compared to Vancouver. This, in turn may be due to the different environments available for burrowing and nest building in the two cities.

Although age was associated with wounding in both cities, wounding was an important risk factor for infection independent of age. There was also a significant interaction between age and wounding, suggesting that impact of wounding on *L. interrogans* transmission is greater in younger animals. Yet, as there is no significant shedding of *L. interrogans* in saliva (Donovan et al., 2018), wounding does not represent a direct route of transmission. Rather, bite wounds may reflect specific social interactions that promote transmission, particularly behaviors associated with dominance and social hierarchies (e.g., fighting). This suggests that, while "random" exposure in the environment may be a source of *L. interrogans* for rats, specific behaviors are also responsible for *L. interrogans* infection. This is important because anthropogenic interventions that alter rat population and social structures, and associated intraspecific interactions, may have unforetold impacts on *L. interrogans* ecology in rats. Indeed, a recent study in Vancouver rats showed that lethal pest control can increase the prevalence of *L. interrogans* in rats (Lee et al., 2018). The results of this study suggest that behavioral drivers of *L. interrogans* infection may be common among cities, therefore those undertaking pest control with the goal of reducing public health threats should do so with the understanding of the potential impacts of different control approaches on rats and the pathogens they carry.

Although social factors may be associated with *L. interrogans* infection in both cities, the specific behaviors and/or interactions that facilitate infection may vary. This possibility is highlighted by the fact that sexual maturity was an important independent risk factor for infection in Salvador but not Vancouver. Vancouver and Salvador also differed significantly with regard to the impact of geography on infection. Specifically, the random variation in the odds of infection by trapping site was much greater in Vancouver than in Salvador. This could be attributed to differences in study design, as trapping was conducted on a finer scale in Vancouver compared to Salvador. The difference in sampling methodology between the two cities, however, was a direct result of differences in the built environment. Specifically, Vancouver's block system allowed a systematic, grid-like approach, which could not be replicated in Salvador. Differences in the built environment may also create differences in rat and pathogen ecology between the two cities. For example, in Vancouver, paved streets heavily used by vehicular traffic separated adjacent city blocks, while in Salvador, trapping points were separated by open spaces and fewer, smaller roadways (i.e., avenues). Genetic evidence suggests that roads may present anthropogenic barriers to rat movement (Richardson et al., 2017; Combs et al., 2018) isolating colonies and creating a markedly heterogeneous pattern of disease distribution even over short geographic distances (Himsworth et al., 2013a, 2015a,b). In the absence of significant roadways, it may be the case that rat colonies co-exist in closer proximity to one another and/or have a greater degree of direct or indirect contact. This, in turn, may facilitate *L. interrogans* transmission among colonies. Indeed, it is of note that the prevalence of *L. interrogans* infection in Vancouver ranged from 0 to 66% among blocks (Himsworth et al., 2013a). It may therefore be the case that colony segregation in Vancouver is partially responsible for both the low overall prevalence of the pathogen and the marked random variation in block-level prevalence.

Limitations

One limitation of this study is that, lacking a way to accurately determine the age of rats, we used a weight-derived estimate as a proxy of age. This could lead to miscalculations of age because weight-based age estimates do not account for rat population characteristics that could cause disparities in rat weight, such as genetics, health status, and nutritional condition. For example, rats in poorer health and with lower nutritional status might weigh less, and would therefore be calculated as younger than a healthy rat of the same age in a nutrient-rich environment. However, as the age estimates of rats leaving the nest for both locations were similar, this suggests that age estimates, at least for younger animals, are aligned. Similarly, while rats in Salvador were significantly heavier than rats in Vancouver, they were also more likely to be sexually mature, suggesting that weight is unlikely to have markedly affected differences in age estimates between the two sites.

A second limitation was the variation in the duration of pre-baiting and active trapping between Salvador and Vancouver which was a result of the collaboration between the two study groups being established after the fieldwork had already been completed. It is not known how variations in pre-baiting time might impact the factors included in this study. However, it has been shown that larger, sexually mature rats are more likely to be captured earlier in the trapping period, compared to smaller and immature rats (Himsworth et al., 2014; Byers et al. in unpublished). Although this would not impact analyses at the level of the individual rat, given that mass is positively associated with *L. interrogans* infection, studies with shorter trapping periods, such as that in Salvador, could produce an inflated estimate of *L. interrogans* prevalence. However, Byers et al. (in unpublished) found no significant association between *L. interrogans* infection status and trap day, suggesting that the impact of trapping period on the results of this study are unlikely to be significant.

Finally, it is important to note that the trapping methodology used in this study would not allow us to estimate rat abundance

or population density in either city. Indeed, there have yet to be any published studies investigating the impact of these factors on *L. interrogans* dynamics in urban rats. Given that many diseases are density-dependent (Begon et al., 2002), and density-dependent transmission may influence the efficacy of control measures, such as population reduction, we suggest that this should be a priority for future study.

CONCLUSIONS

Overall, this study highlights the similarities and differences in ecology of *L. interrogans* in rat populations in two ecologically distinct urban environments. We reveal that both increasing age and number of bite wounds heighten the odds that a rat will acquire *L. interrogans,* and that rats are more likely to leave the nest with infection in Salvador than in Vancouver. Differences between the study areas in pathogen prevalence and spatial heterogeneity of infected rats further suggests that both the local environment and rat social structures/behaviors are important drivers in transmission events. These nuances underscore the importance of geographical location in *L. interrogans* ecology, and reiterate the necessity of understanding rat-pathogen dynamics both globally and locally to devise effective mitigation techniques. Priorities for future studies include developing and/or validating accurate tools for aging urban rats, as well as repeating these analyses in other urban ecosystems, both similar and different to those included here, in order to determine whether these findings can be supported and/or expanded upon.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

This study was conceived of by AM, CH, and FC. Sample collection design was conceived and developed by FC, AK, JC, and CH. Sample collection was performed by FC and CH. Data analysis was performed by AM and CH. Figures were created by AM and KB. Writing of the manuscript was performed by CH, KB, and AM with revisions provided by FC. Funding was acquired by CH and FC.

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REFERENCES

Aviat, F., Blanchard, B., Michel, V., Blanchet, B., Branger, C., Hars, J., et al. (2009). *Leptospira* exposure in the human environment in France: a survey in feral rodents and in fresh water. *Comp. Immunol. Microb. Infect. Dis*. 32, 463–476. doi: 10.1016/j.cimid.2008.05.004

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Begon, M., Bennett, M., Bowers, R. G., French, N. P., and Hazel, S. M. (2002). A clarification of transmission terms in host-microparasite models: numbers, densities and areas. *Epidemiol. Infect.* 129, 147–153. doi: 10.1017S0950268802007148

Burthe, S. J., Lambin, X., Telfer, S., Douglas, A., Beldomenico, P., Smith, A., et al. (2010). Individual growth rates in natural field vole, *Microtus agrestis*, populations exhibiting cyclic population dynamics. *Oecologia* 162, 653–661. doi: 10.1007/s00442-009-1495-6

Calhoun, J. B. (1963). *The Ecology and Sociology of the Norway Rat*. Bethesda, MD: US Department of health, Education and Welfare, Bethesda.

Casanovas-Massana, A., Costa, F., Riediger, I. N., Cunha, M., De Oliveira, D., Mota, D. C., et al. (2018). Spatial and temporal dynamics of pathogenic *Leptospira* in surface waters from the urban slum environment. *Water Res.* 130, 176–184. doi: 10.1016/j.watres.2017.11.068

Combs, M., Byers, K. A., Ghersi, B. M., Blum, M. J., Caccone, A., Costa, F., et al. (2018). Urban rat races: spatial population genomics of brown rats (*Rattus norvegicus*) compared across multiple cities. *Proc. R. Soc. B.* 285, 20180245–20180210. doi: 10.1098/rspb.2018.0245

Costa, F., Carvalho-Pereira, T., Begon, M., Riley, L., and Childs, J. (2017). Zoonotic and vector-borne diseases in urban slums: opportunities for intervention. *Trends Parasitol.* 33, 660–662. doi: 10.1016/j.pt.2017.05.010

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., et al. (2015a). Global morbidity and mortality of leptospirosis: a systematic review. *PLoS. Negl. Trop. Dis*. 9:e0003898. doi: 10.1371/journal.pntd.0003898

Costa, F., Porter, F. H., Rodrigues, G., Farias, H., de Faria, M. T., Wunder, E. A., et al. (2014a). Infections by *Leptospira interrogans*, Seoul Virus, and *Bartonella* spp. among Norway rats (Rattus norvegicus) from the urban slum environment in Brazil. *Vector Borne Zoo Dis*. 14, 33–40. doi: 10.1089/vbz.2013.1378

Costa, F., Ribeiro, G. S., Felzemburgh, R. D., Santos, N., Reis, R. B., Santos, A. C., et al. (2014b). Influence of household rat infestation on *Leptospira* transmission in the urban slum environment. *PLoS. Negl. Trop. Dis*. 8:e3338. doi: 10.1371/journal.pntd.0003338

Costa, F., Wunder, E. A., De Oliveira, D., Bisht, V., Rodrigues, G., Reis, M. G., et al. (2015b). Patterns in *Leptospira* shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission. *PLoS. Negl. Trop. Dis*. 9:e0003819. doi: 10.1371/journal.pntd.0003819.

De Oliveira, D., Figueira, C. P., Zhan, L., Pertile, A. C., Pedra, G. G., Gusmão, I. M., et al. (2016). *Leptospira* in breast tissue and milk of urban Norway rats (*Rattus norvegicus*). *Epidemiol Infect*. 144, 2420–2429. doi: 10.1017/S0950268816000637

Donovan, C. M., Lee, M. J., Byers, K. A., Bidulka, J., Patrick, D. M., and Himsworth, C. G. (2018). Leptospira spp. in the oral cavity of urban brown rats (Rattus norvegicus) from Vancouver, Canada—Implications for rat-rat and rat-human transmission. *J. Wildl. Dis*. 54, 635–637. doi: 10.7589/2017-08-194

Easterbrook, J. D., Kaplan, J. B., Vanasco, N. B., Reeves, W. K., Purcell, R. H., Kosoy, M. Y., et al. (2007). A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol. Infect*. 135, 1192–1199. doi: 10.1017/S0950268806007746

Evangelista, K. V., and Coburn, J. (2010). *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiol*. 5, 1413–1425. doi: 10.2217/fmb.10.102

Felzemburgh, R. D., Ribeiro, G. S., Costa, F., Reis, R. B., Hagan, J. E., Melendez, A. X., et al. (2014). Prospective study of leptospirosis transmission in an urban slum community: Role of poor environment in repeated exposures to the *Leptospira* agent. *PLoS. Negl. Trop. Dis*. 8:e2927. doi: 10.1371/journal.pntd.0002927

Hagan, J. E., Moraga, P., Costa, F., Capian, N., Ribeiro, G. S., Wunder, E. A., et al. (2016). Spatiotemporal determinants of urban leptospirosis transmission: Four-year prospective cohort study of slum residents in Brazil. *PLoS. Negl. Trop. Dis*. 10:e0004275. doi: 10.1371/journal.pntd.0004275

Hartskeerl, R. A., Collares-Pereira, M., and Ellis, W. A. (2011). Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin. Microbiol. Infect*. 17, 494–501. doi: 10.1111/j.1469-0691.2011.03474.x

Himsworth, C. G., Bai, Y., Kosoy, M. Y., Wood, H., DiBernardo, A., Lindsay, R., et al. (2015a). An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul Hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: Is pathogen presence a reflection of global and local rat population structure? *Vector Borne Zoo. Dis*. 15, 21–26. doi: 10.1089/vbz.2014.1657

Himsworth, C. G., Bidulka, J., Parsons, K. L., Feng, A. Y., Tang, P., Jardine, C. M., et al. (2013a). Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. *PLoS. Negl. Trop. Dis*. 7:e2270. doi: 10.1371/journal.pntd.0002270.s001

Himsworth, C. G., Jardine, C. M., Parsons, K. L., Feng, A. Y., and Patrick, D. M. (2014). The characteristics of wild rat (*Rattus* spp.) populations from an inner-city neighborhood with a focus on factors critical to the understanding of rat-associated zoonoses. *PLoS ONE* 9:e91654. doi: 10.1371/journal.pone.0091654

Himsworth, C. G., Parsons, K. L., Jardine, C., and Patrick, D. M. (2013b). Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne Zoo. Dis*. 13, 349–359. doi: 10.1089/vbz.2012.1195

Himsworth, C. G., Zabek, E., Desruisseau, A., Parmley, E. J., Reid-Smith, R., Jardine, C. M., et al. (2015b). Prevalence and characteristics of *Escherichia coli* and *Salmonella* spp. in the feces of wild urban Norway and black rats (*Rattus norvegicus and Rattus rattus*) from an inner-city neighborhood of Vancouver, Canada. *J. Wildl. Dis*. 51, 589–600. doi: 10.7589/2014-09-242

Jensen, P. M., and Magnussen, E. (2016). Is it too cold for *Leptospira interrogans* transmission on the faroese islands? *Infect. Dis.* 48, 156–160. doi: 10.3109/23744235.2015.1092579

Johnson, M. A., Smith, H., Joseph, P., Gilman, R. H., Bautista, C. T., Campos, K. J., et al. (2004). Environmental exposure and leptospirosis, Peru. *Emerg. Infect. Dis*. 10, 1016–1022. doi: 10.3201/eid1006.030660

Koizumi, N., Muto, M., Tanikawa, T., Mizutani, H., Sohmura, Y., Hayashi, E., et al. (2009). Human leptospirosis cases and the prevalence of rats harbouring *Leptospira interrogans* in urban areas of Tokyo, Japan. *J. Med. Microbiol*. 58, 1227–1230. doi: 10.1099/jmm.0.011528-0

Krøjgaard, L. H., Villumsen, S., Markussen, M. D. K., Jensen, J. S., Leirs, H., and Heiberg, A. C. (2009). High prevalence of *Leptospira* spp. in sewer rats *(Rattus norvegicus). Epidemiol. Infect*. 137, 1586–1592. doi: 10.1017/S0950268809002647

Lee, M. J., Byers, K. A., Donovan, C. M., Bidulka, J. J., Stephen, C., Patrick, D. M., et al. (2018). Effects of culling on *Leptospira interrogans* carriage by rats. *Emerg. Infect. Dis*. 24, 356–360. doi: 10.3201/eid2402.171371

Maas, M., De Vries, A., Reusken, C., Buijs, J., Goris, M., Hartskeerl, R., et al. (2018). Prevalence of *Leptospira* spp. and Seoul hantavirus in brown rats (*Rattus norvegicus*) in four regions in the Netherlands, 2011-2015. *Infect. Ecol. Epidemiol*. 8:1490135. doi: 10.1080/20008686.2018.1490135

McVea, D. A., Himsworth, C. G., Patrick, D. M., Lindsay, L. R., Kosoy, M., and Kerr, T. (2018). Exposure to rats and rat-associated *Leptospira* and *Bartonella* species among people who use drugs in an impoverished, inner-city neighborhood of Vancouver, Canada. *Vector Borne Zoo Dis*. 18, 82–88. doi: 10.1089/vbz.2017.2179

Minter, A., Diggle, P. J., Costa, F., Childs, J., Ko, A. I., and Begon, M. (2017). Evidence of multiple intraspecific transmission routes for *Leptospira* acquisition in Norway rats (*Rattus norvegicus*). *Epidemiol. Infect*. 70, 1–11. doi: 10.1017/S0950268817002539

Puckett, E. E., Park, J., Combs, M., Blum, M. J., Bryant, J. E., Caccone, A., et al. (2016). Global population divergence and admixture of the brown rat (*Rattus norvegicus*). *Proc. R. Soc. B*. 283:20161762. doi: 10.1098/rspb.2016.1762

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/

Richardson, J. L., Burak, M. K., Hernandez, C., Shirvell, J. M., Mariani, C., Carvalho-Pereira, T. S., et al. (2017). Using fine-scale spatial genetics of Norway rats to improve control efforts and reduce Leptospirosis risk in urban slum environments. *Evol. Appl*. 10, 323–337. doi: 10.1111/eva.12449

Rothenburger, J. L., Himsworth, C. G., Clifford, C. B., Ellis, J., Treuting, P. M., and Leighton, F. A. (2015a). Respiratory pathology and pathogens in wild urban rats (*Rattus norvegicus* and *Rattus rattus*). *Vet. Pathol*. 52, 1210–1219. doi: 10.1177/0300985815593123

Rothenburger, J. L., Himsworth, C. G., Treuting, P. M., and Leighton, F. A. (2015b). Survey of cardiovascular pathology in wild urban *Rattus norvegicus* and *Rattus rattus*. *Vet Pathol* 52, 201–208. doi: 10.1177/0300985814528220

Santos, N. J., Sousa, E., Reis, M. G., Ko, A. I., and Costa, F. (2017). Rat infestation associated with environmental deficiencies in an urban slum community with high risk of leptospirosis transmission. *Cad Saúde Pública* 33:e00132115. doi: 10.1590/0102-311x00132115

Scialfa, E., Bolpe, J., Bardón, J. C., Ridao, G., Gentile, J., and Gallicchio, O. (2010). Isolation of *Leptospira interrogans* from suburban rats in Tandill, Buenos Aires, Argentina. *Rev. Argent Microbiol*. 42, 126–128. doi: 10.1590/S0325-75412010000200012

Smith, H. A. (2000). *Where Worlds Collide: Social Polarisation at the Community Level in Vancouver's Gastown/Downtown Eastside*. The University of British Columbia, Vancouver, Canada.

Stoddard, R. A., Gee, J. E., Wilkins, P. P., McCaustland, K., and Hoffmaster, A. R. (2009). Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagn. Microbiol. Infect. Dis.* 64, 247–255. doi: 10.1016/j.diagmicrobio.2009.03.014.

Thiermann, A. B. (1977). Incidence of leptospirosis in the Detroit rat population. *Am. J. Trop. Med. Hyg*. 26, 970–974.

Vanasco, N. B., Sequeira, M. D., Sequeira, G., and Tarabla, H. D. (2003). Associations between leptospiral infection and seropositivity in rodents and environmental characteristics in Argentina. *Prev. Vet. Med*. 60, 227–235. doi: 10.1016/S0167-5877(03)00144-2

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Unbiased Sampling for Rodents and Other Small Mammals: How to Overcome Neophobia Through Use of an Electronic-Triggered Live Trap—A Preliminary Test

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Live-trapping of urban rodents and other small mammals poses several challenges for researchers and pest control professionals (PMPs). Most traps are novel to the natural environment and elicit neophobic, or trap-shy, behaviors. Thus, animals captured in traditional traps may either be the least risk-averse, or most desperate. Consequently, individuals of the lowest social ranks, those in poorest health, and the slowest learners are more likely to be captured. This is problematic for research because non-random samples may lead to over-generalization and false conclusions. To address these problems, we developed an inconspicuous, hanging live-trap prototype. In addition to being hard to detect, the trap enables setting several parameters of animal movement and detection before the trap is triggered. The neophobic reaction could then be significantly reduced because animals do not need to enter any trap-like objects—instead they move and feed on a familiar surface. At a time predefined by the user, the triggering mechanism drops a transparent cover over the animal, preventing it from fleeing and enabling either transport to the laboratory, mark-and-release or disposal. Here, we report our initial purpose, design and preliminary results. Animals triggered the trap 34 times during our 1-month preliminary assessment. During this time, 32 individuals were captured (25 Norway rats and 7 house mice) for a 94% catch rate. Video surveillance revealed no obvious signs of non-random sampling as all trapped rats were representative of a broad range of sizes and ages. There were no signs of low social status (e.g., scar markings, parasitism, or poor health). Importantly, we found a low latency following capture, as released animals showed no instances of increased exploratory or cautious behaviors such as rearing or sniffing, near the hidden traps. More monitoring of this design is needed before future conclusions can be drawn. However, these results should encourage a full range of experimental trials from neuroscientists, urban ecologists, pest control professionals (PMPs) and conservationists who seek randomized samples or who work with trap-shy species.

Keywords: arduino, lab-rat model, neophobia, random-sampling, trapping, trap-shyness, randomization, wild rat

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INTRODUCTION

Safe, random trapping of live animals is important for research (Calhoun, 1962; Lockard, 1968; Stryjek and Pisula, 2008; Stryjek et al., 2012) and the pest control industry (Macdonald et al., 1999; Mason and Littin, 2003; Littin et al., 2014) alike. However, the use of traditional trap designs has compromised our ability to collect truly random samples of a given species (Mitchell, 1976; Stryjek and Pisula, 2008; Stryjek et al., 2012). For ecologists, trap-and-release studies are essential for accurately estimating the size of a population (Chao, 1989). Conservation biologists trap prior to translocating flocks or herds (Griffith et al., 1989), this is particularly important to do in the case of reintroductions (Abbott, 2000; de Milliano et al., 2016) or when training threatened animals to be "wary" of a novel predator (Blumstein et al., 2002). Psychologists and neuroscientists who use rat models in the laboratory or in the field, may trap wild rats, or even create new strains of outbred laboratory rats to increase variability in their study populations (Stryjek and Pisula, 2008). Epidemiologists and public health officials may trap rodents as part of surveillance programs to determine if, or when, potential pathogens are entering the population (Firth et al., 2014; Frye et al., 2015; Parsons et al., 2015, 2016).

Random live-trapping serves two essential functions. The first rule of warfare is to "know thy enemy" (Macdonald et al., 1999; Singleton et al., 2007). Thus, wildlife researchers and rodentologists must use highly-variable, random samples of a population to fully understand the efficacy of attractants, repellents and learning/appeasement tools on a pest species (Parsons et al., 2017, 2018). Finally, pest management professionals (PMPs) may routinely trap animals as part of their control efforts (Himsworth et al., 2013). Unfortunately trapping rarely removes the healthy, reproductive members of a population. Instead it is biased by sex, age or social status (Firth et al., 2014; Parsons et al., 2015). Pest trapping is particularly likely in food manufacturing facilities where it is often illegal to introduce poisons, or kill-traps (snap traps or glue traps). The latter is an issue because animal carcasses may accumulate along with potential pathogens as well as carry poisons from other areas, whereas glue traps have also fallen out of favor for ethical reasons. In addition, live-traps are often chosen for aesthetic and ethical reasons (many people refuse to kill animals, even if done humanely; Mason and Littin, 2003; Powell and Proulx, 2003). Finally, live-traps are used when protected species become pests locally [e.g., the Beech marten; *Martes foina;* Rondinini and Boitani, 2002].

However, despite the broadscale need in research and pest control to trap random samples, live-trapping continues to be limited and biased toward age, social status or sex (Firth et al., 2014; Parsons et al., 2015). This is especially surprising given the lack of emphasis made on other field based technologies such as improved camera trapping techniques (Norouzzadeh et al., 2018). To date, several live-trapping methods, of varying levels of efficacy, have been designed and implemented (Corrigan and Moreland, 2001). In the case of rodents, the most popular methods include various kinds of box-style wire mesh or traps made of full metal sheets, such as Sherman, Tomahawk and Havahart traps (Hice and Velazco, 2013). Triggering mechanisms are almost always placed inside and must be stepped on or pulled with some force in order to close the trap. Such traps have either one or two entrances. Anthony et al. (2005) compared large and small Longworth and Sherman traps for efficacy in trapping long tailed shrews (*Sorex dispar*) and western harvest mice (*Reithrodontomys megalotis*). While this study focused on the number of animals of each species caught over 3 years, it did not consider whether either trap was more likely to capture a representative sample of the variation in each population. Sampling bias for each trap-type was examined for pit traps, funnel traps and Elliot traps in Western Australia (Thompson and Thompson, 2007). However, these trap designs varied by species-size (herpetofauna and small mammals), and there was no examination of within-species variability. In a rare study in 2002, Jacob et al. (2002) contrasted the performance of two trap-types (Ugglan and Longworth) in field studies of house mice (*Mus musculus*) in southeastern Australia. Both traps performed similarly, however, Ugglan traps were more likely to attract males. These trials did not relate inter-species variability other than sex bias. In another Australian study Stokes (2013) compared enclosed Elliot traps to open wire cage traps in trapping black rats (*Rattus rattus*) and the native rat (*Rattus fuscipes*). Due to the biases of each type toward species, life stage or sex, the author recommended multiple trap types be used together.

Indeed, trap size rather than trap type (Ribeiro-Júnior et al., 2011) or intra-species variability has been the most examined aspect of trapping over the last 30 years. Many of the remaining trap studies have focused on humane traps to minimize wildlife suffering from capture (Olsen et al., 1988).

Multiple catch traps (e.g., Monarch Design) are less commonly used; in this case, animals enter the trap through a funnel entrance which leads to a weighted trapdoor (Vanderduys, 2016). Other live-trapping methods of small mammals are pitfalls, artificial burrows and nest boxes (Sikes et al., 2011). Yet these approaches are decades old and continue to generate biased catches (Zentall and Levine, 1972).

The general problem with most traps is that, as novel objects, they elicit neophobic reactions (Barnett, 1958; Calhoun, 1962; Mitchell, 1976) which may sometimes persist for weeks, with some instances of animals never approaching the object (RS observation). In addition, after several individuals from a given population are trapped, other individuals start avoiding the traps via social facilitation (Zentall and Levine, 1972), which makes those traditional trapping methods suitable for trapping only a sub-sample of less risk averse or slowest learning animals in a given population (Thompson, 1953). Due to the rats' inherent cautious behavior, trap-shyness and ability to develop escape techniques, live-traps are sometimes thought to be impractical and ineffective (Corrigan and Moreland, 2001). Finally, the commonly used triggering mechanisms in traditional designs are susceptible to accumulation of dirt and mechanical defects, which makes the use of classic live-traps even more challenging (RS, MHP personal observations).

Consequently, traditional trapping methods are not only ineffective, they usually do not provide a representative sample of animals(Lockard, 1968; Boice, 1971a). It is well known that individuals of the lowest social ranks, the slowest learners and

those in poor health are far more likely to be captured (Lockard, 1968; Boice, 1971a). The latter is of crucial importance for the scientific research because non-random samples could result in false conclusions and over-generalizations (Burnham and Overton, 1978). When only a small number of individuals are caught, such as the most resilient or least shy, it further increases the risk that these individuals are genotypically unique. When captured for laboratory settings, this may cause a phenomenon called the founder effect, which leads to genetic drift toward features rarely present in the original population (Lande, 1976). From a pest management perspective, PMPs who routinely trap rats may be removing the lowest ranking members (e.g., beta through omega) while having no negative impact on the alphas (Lockard, 1968; Boice, 1971a). Due to the time-cost inefficiency and the above-outlined difficulties, and due to a constant need to capture live wild rats for the purposes of research and breeding in the WWCPS colony (Stryjek and Pisula, 2008), we developed a relatively simple and potentially highly effective rodent trapping technique. It is hoped that by following modifications to the size and weight, this design could possibly be used for capturing other species (e.g., feral cats; Parsons et al., 2018) or even such trap-shy species as the beech marten (Rondinini and Boitani, 2002). The device prototype has no commercial equivalent available on the market, and thus a design such as this may be necessary for research and industry alike.

TRAP DESIGN

The following description aims to facilitate the construction and modification, as well as the implementation of the presented trapping algorithm to other kinds of live-traps. The main part of the trap prototype is an inconspicuous, suspended transparent plastic cover, which is dropped onto the surface to safely capture and prevent the animal from escaping (**Figure 1** and **Supplemental Video 1**). The main advantage of this approach is that animals are not forced to enter the trap. Instead, they can move on a familiar surface, which significantly decreases neophobic reactions (Stryjek and Modlinska, 2016). The transparent cover (L = 59.5 cm, W = 39.5 cm, H = 16.9 cm; Plast Team Basic 30 L) is suspended 20 cm above the ground. On the inside, it is equipped with a motion detector with regulated range and sensitivity. The transparent cover is fixed to a trigger lock with a chain. The trap is operated by an Arduino computer (Barrett, 2013) placed on a rack with a screen and keypad. The latter makes it possible to set an algorithm for the release operation (i.e., dropping of the transparent cover) based on the readings of the PIR (Passive Infra-Red) sensor. The user can set the number of alert cycles and their duration, and thereby set the conditions for triggering the trap. The algorithm (see **Figure 2** and **Supplemental Data Sheet 1**) enables *pre-baiting* (Chitty and Kempson, 1949; Babinska and Bock, 1969). i.e., consuming the bait without operating the trap, which enables rats to roam, forage and interact under the transparent cover and gradually become habituated to any novelty such as the scent of the plastic or the electronics, as these factors are not typically associated with any aversive stimuli (Selden et al., 1991). This is crucial, as at the initial stages of contact with the bait, the rats exhibit a high level of caution and are prepared to flee rapidly.

Among rats, much of tactile stimulation and information about the surrounding environment comes from vibrissae and guard hairs (Vincent, 1912). Therefore, the principle design of the proposed trap is composed of a suspended transparent cover hanging out of sight above the surface. Most animals adapted to nocturnal conditions, such as rodents, have reduced vision (Crawford, 1934). Thus, the transparent cover is very difficult to detect. This allows the researcher or PMP to place the device in targeted areas of known activity. Such as on paths frequently chosen by the animals (rats display strong tendencies to continuously use the same pathways—see Calhoun, 1962). Placing bait on a pathway, along a wall, which is a part of familiarized and relatively safe territory increases the chance of the trap being successful. It is necessary, however, for the user to be able to set a condition that the sensor needs to be activated for several seconds to prevent the trap from being triggered by animals passing (without stopping) beneath the transparent cover.

To better conceal the device, it may not only be placed on the prototype rack, but also be suspended from a ceiling or attached to a wall. The triggering algorithm can also be used in a similar manner to that of other classic types of traps (**Figure 3**) allowing for prebaiting and capturing lightweight (15–20 g) animals such as e.g., mice, shrews and birds that may not be heavy enough to set off a trip pan. A Norway rat (*Rattus norvegicus*) in comparison may average 300 g (Parsons et al., 2017). As for the bait used, rats should not be able to take it away. This why the bait should be viscous and smeared on the floor (RS, MHP observations). To increase attractiveness of the bait, sex hormones and pheromones may also be applied (Takács et al., 2016). Recent data suggests that such application significantly improves trapping success among rats and mice (Takács et al., 2016; Musso et al., 2017). Another way of facilitating captures is generating species-specific auditory cues, which may increase the attraction for animals (Takács et al., 2016).

METHODS

Study Site

This preliminary study was conducted over 1 month (September, 2015) on a wild living colony of Norway rats (*Rattus norvegicus*) on a farm consisting of 4 separate, distant buildings separated from one another by ∼30 m, situated on the outskirts of Warsaw, Poland (52.13°N, 21.00°E) The farm was inhabited by at least two rat colonies. The size of the tested colony had not been controlled for approximately the last 3 years prior to the experiment—neither by means of poisons, nor mechanically. The second colony was not utilized in order to avoid any possible interference or interruption with other behavioral tests conducted on the property (Modlinska and Stryjek, 2016; Stryjek et al., 2018). Cocoa-peanut butter (Nutella®) was used as bait, and it was smeared on the surface directly underneath the transparent cover. The test was conducted in three separate trapping spots placed in three different rooms on the farm.

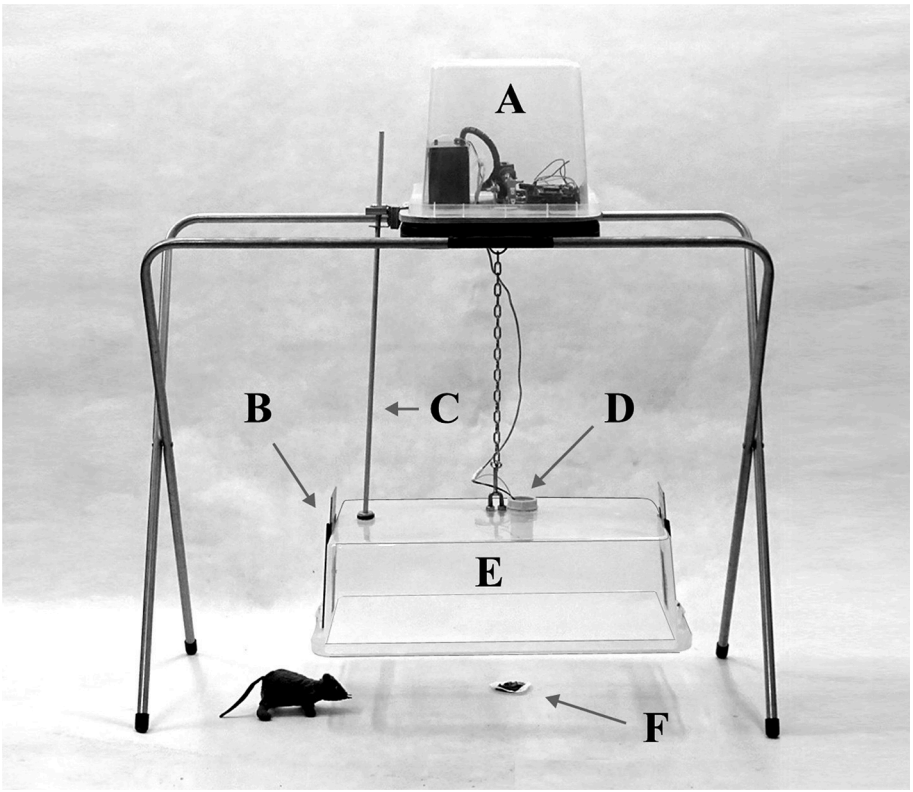

FIGURE 1 | The tested prototype of the trap: **(A)** Arduino computer and triggering mechanism; **(B)** upward-sliding entrance gate used for taking the animals out of the trap; **(C)** bar preventing the transparent cover from moving and rotating; **(D)** motion detector with regulated range and sensitivity; **(E)** falling transparent cover made of plastic; **(F)** bait.

Placement

The surface or floor under the trap prototype should be flat and solid to prevent animals from escaping by digging burrows. Another possible way to prevent escapes is to put a metal wire mesh on the ground or to place it underground and cover it with a thin layer of soil. However, if there are concerns that animals try to dig through the wire, then a plexiglass plate covered with soil may be substituted. Importantly, when this device is utilized outdoors in unpredictable or inclement weather, we recommend either using (or installing) a wind break 3 weeks prior to trapping or making changes to the construction of the rack, causing it to be more firmly situated without swinging. Another option might be to construct a composite weighted rack that limits swinging, however, the researchers would have to account for the fact that a weighted rack could then cause harm to animal when it drops. Therefore, for animal ethics concerns, a heavier outer cover might be fixed in place and subsequently release an internal, but lighter, cover. The wind break approach would not have any impact on trap shy animals, whereas the latter choice includes a physical structure and might require prebaiting alongside the rack. The choice would be up to the reader and the local context of presentation. We recommend these traps be remotely monitored, because unlike normal traps, food, water and shelter will be unavailable unless the end-user makes modifications to the design.

To safely and reliably remove captured animals from the trap, we rely on a phenomenon called thigmotaxis (a tendency to hide in small spaces; Treit and Fundytus, 1988; Stryjek and Modlinska, 2013). Captured rodents will almost always try to flee from the trap. We take advantage of this tendency by transferring them to cardboard boxes, from which they can be moved into transport cages (Stryjek and Modlinska, 2013). After placing a cardboard box next to one of the gate entrances at the sides of the transparent cover, the gate may be opened. The animal, seeking a way to flee, will escape into the adjacent box, in which it can then be transported to a different place (**Supplemental Video 2**).

Except for the instance when the transparent cover is released and dropped, the device operates in absolute silence—neither the PIR sensor, nor the other electronic elements emit sounds (they have been tested with an ultrasound detector—Avisoft-UltraSoundGate 116Hb). The construction of the prototype, as well as a circuit diagram are demonstrated in **Figure 4**. The cost of parts used in the construction is relatively low (<$75) and would be much smaller if mass produced. Though, this expense, even at its most expensive point, could be justified against the overall benefit of highly-variable animal models in the research laboratory (Stryjek and Pisula, 2008), the potential for reduced risk of disease in the wild (Firth et al., 2014; Frye et al., 2015), and the billion-dollar worldwide industry of rodent control (Parsons et al., 2015).

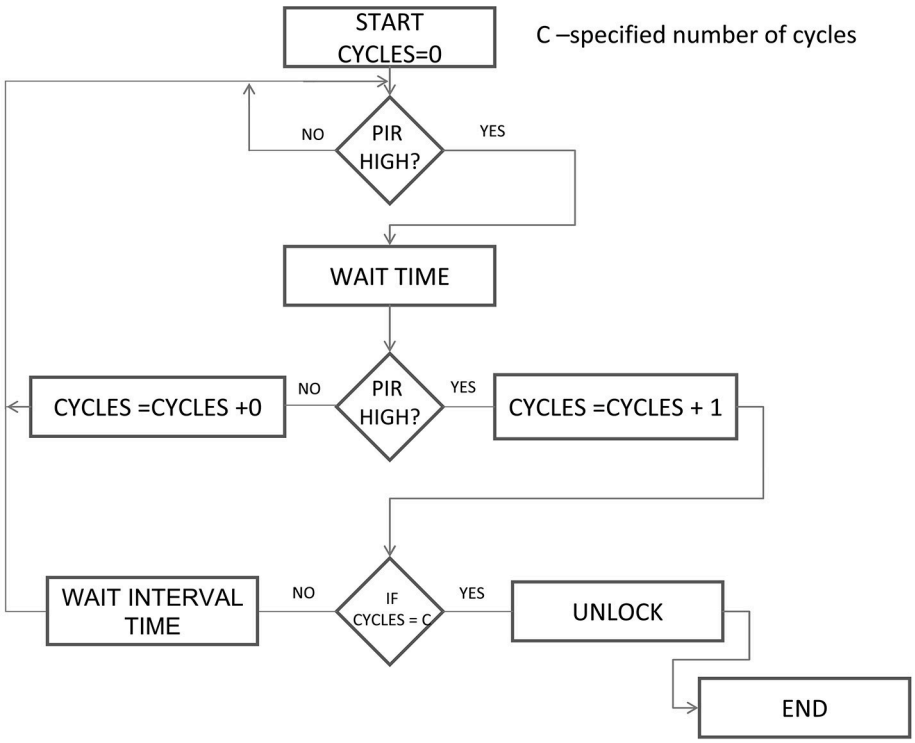

FIGURE 2 | Block diagram showing the operation of the trap.

Assessment Phase

We did not have any set duration for the initial assessment, our only intentions were to (1). establish a catch rate of the animals in a single colony, and (2). determine whether a random sample of animals from a population were captured. Our measure of success was to catch a range of males, females, juveniles, adults. Most importantly, we wanted to determine if healthy individuals or potential high socially ranking animals (no scars or obvious parasitism) were being captured as frequently as sick animals. Lastly, we were interested in determining the latency for repeated capture of animals or repeated use of target areas by previously captured animals. One important aspect that simplified our assessment was knowing that we did not have to mark animals prior to their participation. Because the trap is non-detectable (the essential aspect of the design), animals are ostensibly unaware that there is any trap present. Thus, following capture, the animal would either continue to use the area, in which case their next possible capture would not have been influenced by the previous capture, or the animal would avoid the area. Either way, repeated captures could not influence our outcomes.

We used 4 IR cameras CMOS EC-832-SCH connected to a DVR recorder (EC-7804T), which enabled 24-h observation. The trap and videos were checked on a daily basis. We focused specifically on testing the triggering mechanism set to release the transparent cover after 5 cycles of sensor activation, each lasting 5 s.

RESULTS

The trap was operated 34 times, and 32 animals were captured (25 Norway rats and 7 house mice) which represents a 94% capture rate of animals that trigger the trap. There were no obvious signs of non-random sampling—all trapped rats appeared to be representative of different sizes and ages. Importantly, there were no signs of reduced social status [e.g., scar markings on tail, rump or neck; see Boice, 1971b] or poor health (porphyrin around eyes, uneven vibrissae and guard hairs, or obvious blood) were observed. A qualitative analysis of the video recordings showed that the transparent cover suspended 20 cm above the ground does not elicit any obvious reaction in animals (there were no observed instances of exploratory behaviors, such as rearing or sniffing). After 2–4 captures, some animals started to exhibit avoidance reactions toward the place of trapping. The animals, however, did not avoid the trap itself—when relocated to a different place within the reach of the same colony, the trap proved effective again. A decreased exploration of a given place was observed also after the trap had been removed to another room, which means that the evoked avoidance reaction was linked to the place, and not to the device itself.

DISCUSSION

We prepared a working prototype to be further developed by other researchers and pest control professionals based on

their own context and needs. Our initial results for a single colony of Norway rats in a peri-urban area near Warsaw (Poland) are promising enough to warrant further experimental trials. The range of individuals within a population captured and the low latency suggests the inconspicuous design of the prototype trap helped overcome trap shyness or neophobia. This phenomenon has been identified as the primary barrier to capturing a random sample of animals (Stryjek and Modlinska, 2016) from a population. The efficiency, 32 animals among 34 triggers (94%) was likely higher than among traditional traps, The low efficiency of traditional traps is well known among researchers and PMPs who commonly return to traps that have been triggered, but are empty. We also note that the cost of parts used for construction was relatively low when compared against the benefits of improved lab animal models (Stryjek and Pisula, 2008) and rodent control (Parsons et al., 2015).

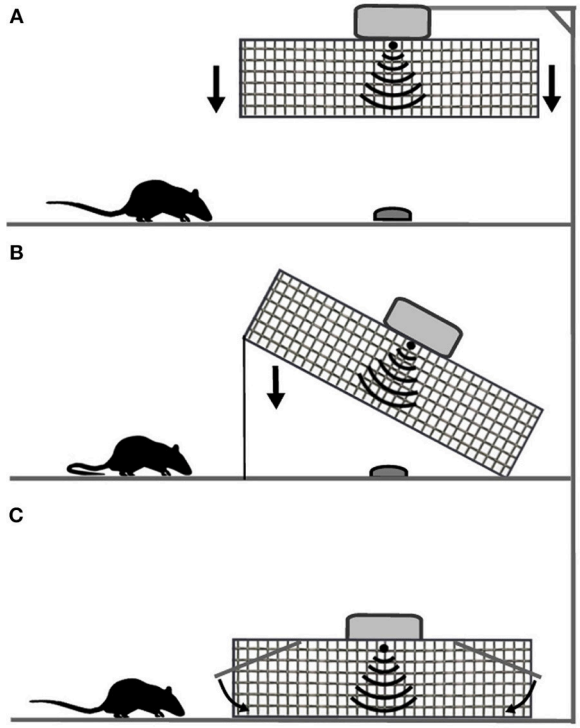

FIGURE 3 | Three ways of implementing the idea: **(A)** falling transparent cover attached to a rack, ceiling or wall; **(B)** classic drop trap; **(C)** classic wire mesh live trap.

The use of a suspended transparent cover meant that it is highly unlikely that the animals would get any sense of a potentially threatening object. This was demonstrated by the low latency of rats approaching the bait with no exploratory behaviors around the trap area. After the trap had been triggered several times, the animals' reaction was to avoid the place where the trap had been placed rather than the trap itself, which when moved elsewhere did not evoke neophobic reaction. Seven non-target animals (*Mus musculus*) were captured. Though we note that further adjustments could have been made to the algorithm in order to limit species to a given weight range. We believe this design is among the first to provide reasonable control over capture of non-target species.

The efficiency of the trap is increased by using an adjustable time of sensor activation and an adjustable number of alert cycles before the triggering mechanism releases the transparent cover—at the initial stages of contact with a new bait, the animals exhibit caution and are prepared to flee rapidly. Moreover, the condition that the sensor needs to be activated for several seconds prevents the trap from being triggered by animals passing through beneath

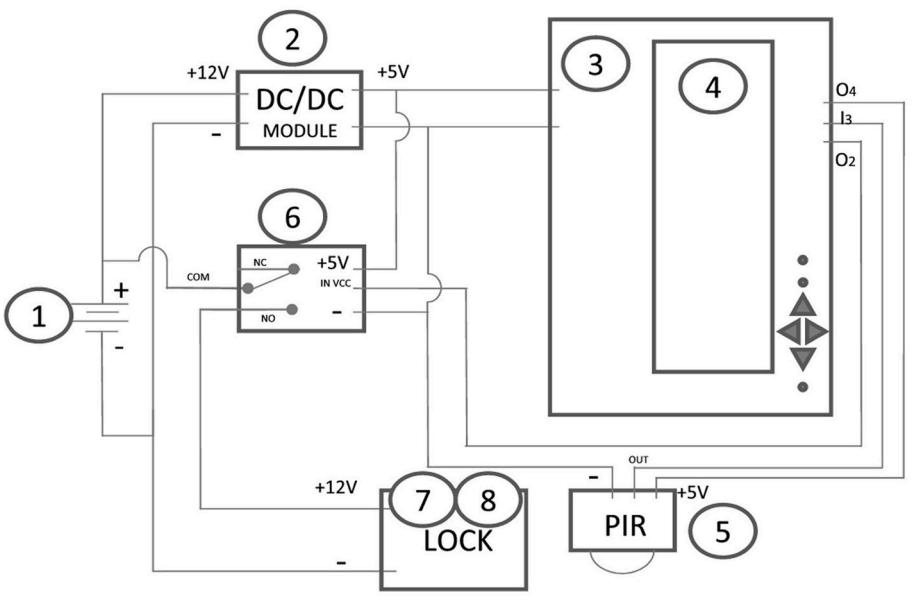

FIGURE 4 | Electric diagram of the trap: (1) battery 12V/7AH; (2) DC LM2596 module; (3) Arduino Leonardo ATmega32u4 R3; (4) LCD keypad shield LCD 1602; (5) PIR HC SR-501; (6) relay; (7) actuator (car central lock actuator); (8) lock (car trunk lock).

the transparent cover, which makes it possible to place the trap on frequently chosen paths.

In our opinion, potential improvements could involve reducing the size of the device, which is relatively large in its prototype version, as well as using a metal wire mesh instead of plastic. A transparent cover construction would improve the airflow, prove more difficult to bite through or lift by any animals trying to escape. The type of research desired will determine which changes should be made. For instance, in some rural or field applications, a shaded cover instead of transparent should be considered. This allows animals some form of shelter. Inside a building however, the transparent cover seems ideal, as it allows for the ease of identification of the type of the target animal.

It seems worthwhile to consider suspending the trap from a ceiling or wall. This is because using the rack in the prototype introduced changes into the space in which the animals moved, thereby potentially triggering a neophobic reaction. We believe another improvement would involve software modification to enable the user to set the time in which the trap would be active (e.g., to trap nocturnal animals and avoid trapping diurnal animals). An improved system could also inform its users about all instances when the trap was triggered via text or multimedia messages (Macdonald et al., 1999; Notz et al., 2017), which would allow an identification of the trapped species and shorten the time the animal is kept inside the trap.

The above-described alterations could help test the efficiency of this presented triggering mechanism in trapping other animal species. Its implementation into other kinds of traps may help improve trapping efficiency (both the number of captured rats, and representative samples among a population) while reducing the risk of accidental capture of non-target animals, without any risk of secondary poisoning or comprised animal safety. While improved techniques to camera trapping are regularly being made (Norouzzadeh et al., 2018), there have been few changes to live traps. We hope other researchers and pest control professionals will utilize and adapt the algorithm to their own needs.

DATA AVAILABILITY STATEMENT

The authors are keen for other researchers to consider or improve upon their design, thus all information is available upon request to RS.

ETHICS STATEMENT

This trap meets or exceeds current requirements for ethical use (Powell and Proulx, 2003; Gannon and Sikes, 2007). Under Polish law, trapping pest species does not require permission of the local ethics committee for animal experimentation. None of the captured animals spent more than 10 h inside the trap. Some of the captured rats were set free on the spot (16%). This type of trap should always be remotely monitored because warm bedding, food, and/or water may not be available unless the end-user customizes the application to their needs.

AUTHOR CONTRIBUTIONS

RS and AK designed the prototype and conducted the study. RS and MP wrote the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00011/full#supplementary-material

Supplemental Video 1 | Video presenting the trap in action.

Supplemental Video 2 | Video presenting the technique of taking captured animals out from the trap.

Supplemental Data Sheet 1 | Trap algorithm.

REFERENCES

Abbott, I. (2000). Improving the conservation of threatened and rare mammal species through translocation to islands: case study Western Australia. *Biol. Conser.* 93, 195–201. doi: 10.1016/S0006-3207(99)00144-5

Anthony, N. M., Ribic, C. A., Bautz, R., and Garland, T. Jr. (2005). Comparative effectiveness of Longworth and Sherman live traps. *Wildlife Soc. Bull.* 33, 1018–1026. doi: 10.2193/0091-7648(2005)33[1018:CEOLAS]2.0.CO;2

Babinska, J., and Bock, E. (1969). The effect of pre-baiting on captures of rodents. *Acta Theriol.* 14, 267–270. doi: 10.4098/AT.arch.69-20

Barnett, S. A. (1958). An analysis of social behaviour in wild rats. *J. Zool.* 130, 107–152. doi: 10.1111/j.1096-3642.1958.tb00565.x

Barrett, S. F. (2013). Arduino microcontroller processing for everyone! *Synth. Lect. Dig. Circuits Syst.* 8, 1–513. doi: 10.2200/S00522ED1V01Y201307DCS043

Blumstein, D. T., Mari, M., Daniel, J. C., Ardron, J. G., Griffin, A. S., and Evans, C. S. (2002). Olfactory predator recognition: wallabies may have to learn to be wary. *Anim. Conser.* 5, 87–93. doi: 10.1017/S1367943002002123

Boice, R. (1971a). Laboratorizing the wild rat (*Rattus* norvegiens). *Behav. Res. Methods Instrument.* 3, 177–182. doi: 10.3758/BF03208127

Boice, R. (1971b). Excessive water intake in captive Norway rats with scar-markings. *Physiol. Behav.* 7, 723–725. doi: 10.1016/0031-9384(71)90139-9

Burnham, K. P., and Overton, W. S. (1978). Estimation of the size of a closed population when capture probabilities vary among animals. *Biometrika* 65, 625–633. doi: 10.1093/biomet/65.3.625

Calhoun, J. (1962). *The Ecology and Sociology of the Norway Rat*. Bethesda, MD: US Public Health Service Publication,

Chao, A. (1989). Estimating population size for sparse data in capture-recapture experiments. *Biometrics* 45, 427–438. doi: 10.2307/2531487

Chitty, D., and Kempson, D. (1949). Prebaiting small mammals and a new design of live trap. *Ecology* 30, 536–542. doi: 10.2307/1932457

Corrigan, R. M., and Moreland, D. (2001). *Rodent Control: a Practical Guide for Pest Management Professionals*. Cleveland, OH: GIE Media

Crawford, S. C. (1934). The habits and characteristics of nocturnal animals. *Q. Rev. Biol.* 9, 201–214. doi: 10.1086/394460

de Milliano, J., Di Stefano, J., Courtney, P., Temple-Smith, P., and Coulson, G. (2016). Soft-release versus hard-release for reintroduction of an

endangered species: an experimental comparison using eastern barred bandicoots (*Perameles gunnii*). *Wildlife Res.* 43, 1–12. doi: 10.1071/WR14257

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P. et al. (2014). Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus* norvegicus in New York City. *MBio* 5:e01933-14. doi: 10.1128/mBio.01933-14

Frye, M. J., Firth, C., Bhat, M., Firth, M., Che, X., Lee, D., Williams, S., and Lipkin, W. (2015). Preliminary survey of ectoparasites and associated pathogens from norway rats in New York City. *J. Med. Entomol.* 52, 253–259. doi: 10.1093/jme/tjv014

Gannon, W. L., and Sikes, R. S. (2007). Guidelines of the American society of mammalogists for the use of wild mammals in research. *J. Mammal.* 88, 809–823. doi: 10.1644/06-MAMM-F-185R1.1

Griffith, B., Scott, J. M., Carpenter, J. W., and Reed, C. (1989). Translocation as a species conservation tool: status and strategy. *Science* 245, 477–480. doi: 10.1126/science.245.4917.477

Hice, C. L., and Velazco, P. M. (2013). Relative effectiveness of several bait and trap types for assessing terrestrial small mammal communities in neotropical rainforest. *Mus. Tex Tech Univ*. 316, 1–15.

Himsworth, C. G., Feng, A. Y., Parsons, K., Kerr, T., and Patrick, D. M. (2013). Using experiential knowledge to understand urban rat ecology: a survey of Canadian pest control professionals. *Urban Ecosyst.* 16, 341–350. doi: 10.1007/s11252-012-0261-4

Jacob, J., Ylönen, H., and Hodkinson, C. (2002). Trapping efficiency of Ugglan traps and Longworth traps for house mice in south-eastern Australia. *Wildlife Res.* 29, 101–103. doi: 10.1071/WR01048

Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30, 314–334. doi: 10.1111/j.1558-5646.1976.tb00911.x

Littin, K., Fisher, P., Beausoleil, N., and Sharp, T. (2014). Welfare aspects of vertebrate pest control and culling: ranking control techniques for humaneness. *Rev. Sci. Tech.* 33, 281–289. doi: 10.20506/rst.33.1.2281

Lockard, R. B. (1968). The albino rat: a defensible choice or a bad habit? *Am. Psychol.* 23:734. doi: 10.1037/h0026726

Macdonald, D. W., Mathews, F., and Berdoy, M. (1999). "The behaviour and ecology of *Rattus* norvegicus: from opportunism to kamikaze tendencies," in *Ecologically Based Rodent Management, ACIAR Monograph,* G. Singleton and H. Leirs (Melbourne, VIC: Brown Prior Anderson), 49–80.

Mason, G., and Littin, K. (2003). The humaneness of rodent pest control. *Anim. Welfare.* 12, 12–37. Available online at: http://hdl.handle.net/10214/4712

Mitchell, D. (1976). Experiments on neophobia in wild and laboratory rats: a reevaluation. *J. Comparat. Physiol. Psychol.* 90:190. doi: 10.1037/h0077196

Modlinska, K., and Stryjek, R. (2016). Food neophobia in wild rats (Rattus norvegicus) inhabiting a changeable environment—a field study. *PLoS ONE* 11:e0156741. doi: 10.1371/journal.pone.0156741

Musso, A. E., Gries, R., Zhai, H., Takács, S., and Gries, G. (2017). Effect of male house mouse pheromone components on behavioral responses of mice in laboratory and field experiments. *J. Chem. Ecol.* 43, 215–224. doi: 10.1007/s10886-017-0819-y

Norouzzadeh, M. S., Nguyen, A., Kosmala, M., Swanson, A., Palmer, M. S., Packer, C. et al. (2018). Automatically identifying, counting, and describing wild animals in camera-trap images with deep learning. *Proc. Natl. Acad. Sci. U.S.A.* 115, E5716–25. doi: 10.1073/pnas.1719367115

Notz, E., Imholt, C., Reil, D., and Jacob, J. (2017). Testing automated sensor traps for mammal field studies. *Wildlife Res.* 44, 72–77. doi: 10.1071/WR16192

Olsen, G. H., Linscombe, R. G., Wright, V. L., and Holmes, R. A. (1988). Reducing injuries to terrestrial furbearers by using padded foothold traps. *Wildlife Soc. Bull.* 16, 303–307.

Parsons, M. H., Apfelbach, R., Banks, P. B., Cameron, E. Z., Dickman, C. R., Frank, A. S. et al. (2018). Biologically meaningful scents: a framework for understanding predator–prey research across disciplines. *Biol. Rev.* 93, 98–114. doi: 10.1111/brv.12334

Parsons, M. H., Banks, P. B., Deutsch, M. A., Corrigan, R. F., and Munshi-South, J. (2017). Trends in urban rat ecology: a framework to define the prevailing knowledge gaps and incentives for academia, pest management professionals (PMPs) and public health agencies to participate. *J. Urban Ecol.* 3:jux005. doi: 10.1093/jue/jux005

Parsons, M. H., Banks, P. B., Deutsch, M. A., and Munshi-South, J. (2018). Temporal and space-use changes by rats in response to predation by feral cats in an urban ecosystem. *Front. Ecol. Evol.* 6:146. doi: 10.3389/fevo.2018.00146

Parsons, M. H., Sarno, R., and Deutsch, M. (2015). Jump-starting urban rat research: conspecific pheromones recruit wild rats into a behavioral and pathogen-monitoring assay. *Front. Ecol. Evol.* 3:146. doi: 10.3389/fevo.2015.00146

Parsons, M. H., Sarno, R. J., and Deutsch, M. A. (2016). A Detailed Protocol to enable safe-handling, preemptive detection, and systematic surveillance of rat-vectored pathogens in the urban environment. *Front. Public Health* 4:132. doi: 10.3389/fpubh.2016.00132

Powell, R. A., and Proulx, G. (2003). Trapping and marking terrestrial mammals for research: integrating ethics, performance criteria, techniques, and common sense. *Ilar J.* 44, 259–276. doi: 10.1093/ilar.44.4.259

Ribeiro-Júnior, M. A., Rossi, R. V., Miranda, C. L., and Ávila-Pires, T. C. (2011). Influence of pitfall trap size and design on herpetofauna and small mammal studies in a Neotropical Forest. *Zoologia* 28, 80–91. doi: 10.1590/S1984-46702011000100012

Rondinini, C., and Boitani, L. (2002). Habitat use by beech martens in a fragmented landscape. *Ecography* 25, 257–264. doi: 10.1034/j.1600-0587.2002.250301.x

Selden, N. R., Everitt, B., Jarrard, L., and Robbins, T. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience* 42, 335–350. doi: 10.1016/0306-4522(91)90379-3

Sikes, R. S., Gannon, W. L., Care, A., and Mammalogists, U. C.,o.t.A.S.o. (2011). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mammal.* 92, 235–253. doi: 10.1644/10-MAMM-F-355.1

Singleton, G. R., Brown, P. R., Jacob, J., and Aplin, K. P. (2007). and Sudarmaji, Unwanted and unintended effects of culling: a case for ecologically-based rodent management. *Integrat. zool.* 2, 247–259. doi: 10.1111/j.1749-4877.2007.00067.x

Stokes, V. L. (2013). Trappability of introduced and native rodents in different trap types in coastal forests of south-eastern Australia. *Aust. Mammal.* 35, 49–53. doi: 10.1071/AM12002

Stryjek, R., Mioduszewska, B., Spaltabaka-Gedek, E., and Juszczak, G. R. (2018). Wild Norway rats do not avoid predator scents when collecting food in a familiar habitat: a field study. *Sci. Rep.* 8:9475. doi: 10.1038/s41598-018-27054-4

Stryjek, R., and Modlinska, K. (2013). A thigmotaxis-based method of recapturing and transporting small mammals in the laboratory. *Lab Anim.* 42:321. doi: 10.1038/laban.328

Stryjek, R., and Modlinska, K. (2016). Neophobia in wild rats is elicited by using bait stations but not bait trays. *Int. J. Pest Manag.* 62, 158–164. doi: 10.1080/09670874.2016.1147622

Stryjek, R., Modlinska, K., and Pisula, W. (2012). Species specific behavioural patterns (digging and swimming) and reaction to novel objects in wild type, Wistar, Sprague-Dawley and Brown Norway rats. *PLoS ONE* 7:e40642. doi: 10.1371/journal.pone.0040642

Stryjek, R., and Pisula, W. (2008). Warsaw wild captive pisula stryjek rats (WWCPS)-establishing a breeding colony of Norway Rat in captivity. *Polish Psychol. Bull.* 39, 67–70. doi: 10.2478/v10059-008-0011-x

Takács, S., Gries, R., Zhai, H., and Gries, G. (2016). The sex attractant pheromone of male brown rats: identification and field experiment. *Angew. Chem. Int. Ed.* 55, 6062–6066. doi: 10.1002/anie.201511864

Thompson, G. G., and Thompson, S. A. (2007). Usefulness of funnel traps in catching small reptiles and mammals, with comments on the

effectiveness of the alternatives. *Wildlife Res.* 34, 491–497. doi: 10.1071/WR06081

Thompson, H. V. (1953). Experimental live trapping of rats, with observations on their behaviour. *Br. J. Anim. Behav.* 1, 96–111. doi: 10.1016/S0950-5601(53)80002-X

Treit, D., and Fundytus, M. (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacol. Biochem. Behav.* 31, 959–962. doi: 10.1016/0091-3057(88)90413-3

Vanderduys, E. (2016). *Mungalla Terrestrial Fauna Survey*. (Canberra, ACT: CSIRO), 1–27.

Vincent, S. B. (1912). *The Functions of the Vibrissae in the Behavior of the White Rat*. Chicago, IL: University of Chicago.

Zentall, T. R., and Levine, J. M. (1972). Observational learning and social facilitation in the rat. *Science* 178, 1220–1221. doi: 10.1126/science.178.4066.1220

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Phylogeography of Invasive Rats in New Zealand

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Two species of invasive rats (*Rattus norvegicus* and *R. rattus*) arrived in New Zealand with Europeans in the mid to late eighteenth and nineteenth century respectively. They rapidly spread across the main islands of New Zealand and its offshore islands, displacing the historically introduced *R. exulans*. Today both species are widespread although the distribution of the sub-dominant *R. norvegicus* is patchy. Tissue samples were obtained from 425 *R. rattus* and 130 *R. norvegicus* across the New Zealand archipelago and neighboring islands. We sequenced a standard 545 base pair section of the mitochondrial D-loop in order to construct a modern phylogeography of the two species and to make inference on historical invasion pathways and spread across the country. We found limited diversity in *R. norvegicus* haplotypes, with two widespread haplotypes across New Zealand and its offshore islands most likely corresponding to two independent invasions, potentially with English and Chinese origins, respectively. In contrast we found widespread diversity in *R. rattus* haplotypes across New Zealand and its offshore islands, most likely corresponding to at least four independent invasions to the main North and South Islands, Great Barrier Island archipelago, and Stewart Island archipelago. The most common *R. rattus* haplogroup was found throughout New Zealand and many of its offshore islands, as well as neighboring islands in the Tasman Sea, and has been documented elsewhere across the Pacific, but with European origins. We also found both geographic partitioning and secondary invasions of haplotypes within the main North and South Island. In addition to distinct haplogroups differing by over three base pairs, which exhibit geographical partitioning suggestive of independent invasion events, for both species we also found instances of single base-pair differences within localities, elevating haplotype diversity. The geographical distribution of pelage color morphs also correlates with haplotype distribution, lending further support to the hypothesis and role of independent invasion events.

Keywords: D-loop, genetics, island, mitochondrial DNA, *rattus*, rodent

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INTRODUCTION

Three species of rats have been introduced to the New Zealand archipelago: *Rattus exulans* (Pacific or Polynesian rat), *R. norvegicus* (Norway or brown rat) and *R. rattus* (ship or black rat). *R. exulans* was introduced by Polynesian settlers in the late thirteenth century (Wilmshurst et al., 2008), while *R. norvegicus* and *R. rattus* were introduced by European explorers and settlers in the mid to late eighteenth and nineteenth century respectively (Atkinson, 1973). Each species rapidly spread

throughout the country, and in turn displaced those rat species which had arrived previous to it (Russell et al., 2014). As of the late twentieth century *R. exulans* was mostly restricted to remote parts of the main islands and a few offshore islands of New Zealand, *R. norvegicus* was distributed patchily across the main islands and some offshore islands of New Zealand, and *R. rattus* was distributed abundantly across the main islands and some offshore islands of New Zealand (Russell and Clout, 2004).

Since the middle of the twentieth century these rat species have been progressively eradicated from the uninhabited offshore islands of New Zealand (Clout and Russell, 2006; Russell and Broome, 2016), and since the start of the twenty-first century controlled widely across the main islands of New Zealand (Brown et al., 2015; Russell et al., 2015). The rat species of European origin (*R. norvegicus* and *R. rattus*) have well-documented dispersal capabilities over water and land (Russell et al., 2005; Abdelkrim et al., 2010), indeed *R. norvegicus* is particularly effective at dispersal by swimming and can cross water gaps of two kilometers, while *R. rattus* can cross gaps of hundreds of meters (Bassett et al., 2016). Population genetic studies have helped determine the putative origin of rats on islands (Robins et al., 2016), and whether rats discovered on islands following eradication are survivors or reinvaders (Russell et al., 2010). Such genetic studies have contributed to improved management of invasive rats across New Zealand.

The population genetics of house mice (*Mus musculus*) has been rigorously assessed across the New Zealand archipelago using a variety of genetic markers (Searle et al., 2009; King et al., 2016; Veale et al., 2018). The population genetics of *R. exulans* has also been assessed, as a proxy for Polynesian migration (Matisoo-Smith et al., 1998). However, to date, there has not been a comprehensive national survey of *R. norvegicus* and *R. rattus* genetic diversity across the entirety of the New Zealand archipelago. Regional studies of genetic diversity in New Zealand have been undertaken, particularly for island groups and adjacent coastlines (Russell et al., 2009, 2010; Abdelkrim et al., 2010; Fewster et al., 2011; Robins et al., 2016). Similar regional studies of *R. norvegicus* and *R. rattus* have also been undertaken internationally (Aplin et al., 2011; Song et al., 2014). Such molecular studies can have a variety of applications, including increasing knowledge of invasive rat taxonomy (Bastos et al., 2011; Conroy et al., 2013), evolutionary biology (Lack et al., 2012; Konečný et al., 2013,), ecology (Theuerkauf et al., 2015; Varudkar and Ramakrishnan, 2015), disease epidemiology (Tollenaere et al., 2012; Brouat et al., 2013; Richardson et al., 2017), management (Kaleme et al., 2011; Kajdacsi et al., 2013; Haniza et al., 2015) and historical biogeography (Tollenaere et al., 2010; Lack et al., 2013; López et al., 2013; Brouat et al., 2014; Colangelo et al., 2015; Berthier et al., 2016). In this work, the first comprehensive national survey of mitochondrial genetic diversity for *R. norvegicus* and *R. rattus* across the New Zealand archipelago and surrounding islands is undertaken.

MATERIALS AND METHODS

Samples

Tissue samples were acquired from New Zealand and surrounding islands from 2001 to 2015 through research collections (i.e., regional studies), opportunistic collection (e.g., road kill), and requests to people or groups undertaking rat trapping (**Supplementary Table 1**). Tissue samples were provided as a by-product of pest control in accordance with the Conservation Act (1987) and thus were exempt from animal ethics committee approval under the New Zealand Animal Welfare Act (1999) 30B.1.b.iii, except where indicated in cited studies. Collections were made with a focus on representative geographic coverage of as many islands as possible on which either rat species was known to be or have been present, and for islands larger than 100,000 hectares representative coverage was sought across those islands (i.e., North, South, Stewart). As well as all outlying islands within the New Zealand Exclusive Economic Zone (Campbell, Chatham and Raoul Islands), samples were also obtained from Macquarie, Lord Howe and Norfolk Islands, and Port Jackson, Sydney (Australia). Tissue samples ranged in quality from freshly caught to degraded by some weeks, but previous work assured us that this did not affect the quality of DNA extraction for the level of molecular resolution we required. Tissue samples were geoindexed precisely with GPS or on large islands approximately to nearby landmarks (variation of a few kilometers). Rat species identification was not always known or accurate, but was invariably confirmed following molecular typing.

Sequencing

DNA was extracted using the DNeasy Tissue Kit (Qiagen), or the High Pure PCR Template Preparation Kit (Roche Diagnostics). The 585 bp amplicon of the D-Loop was amplified with the primers EGL4L and RJ3R (Robins et al., 2007). The reaction volume was 20 μL comprising: 10 mM Tris HCl pH 8.3; 50 mM KCl, 2.5 mM $MgCl_2$, primers at 0.5 μM each, dNTPs at 0.15 mM each; 0.5 U of *Taq* polymerase, 1 μL of DNA template. The PCR (polymerase chain reaction) regime was an initial denaturation step of 94°C for 2 min; 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min with a final extension step of 72°C for 5 min. PCR products were visualized and quantified, using a low mass ladder for comparison, on ethidium bromide stained 1% agarose gels. PCR products were purified with ExoSAP-IT (Affymetrix, Inc.). Sequencing was carried out at the Massey University Genome Service, Palmerston North, New Zealand using the BigDye Terminator version 3 sequencing kit, the GeneAmp PCR System 9700 and a capillary ABI3730 DNA analyser, all from Applied Biosystems.

Analysis

The raw sequences were trimmed, edited, aligned, and grouped into haplotypes using the software package SEQUENCHER (Gene Codes). The relationships among the haplotypes were estimated with a minimum spanning haplotype network (Bandelt et al., 1999) as implemented in PopART (http://popart.otago.ac.nz).

RESULTS

A total of 23 unique haplotypes were found and lodged in GenBank (**Table 1**).

TABLE 1 | The number of unique haplotypes across New Zealand and surrounding islands with GenBank accession numbers.

Haplotype	*n*	Location	GenBank accession
Norhap01	87	North Island and neighboring islands; Raoul Island; Chatham Island; Campbell Island; township of Oban (on Stewart Island)	MH751483
Norhap02	39	South Island; Stewart Island	MH751484
Norhap03	1	Township of Bluff on South Island	MH751485
Norhap04	1	Rare on North Island	MH751486
Norhap05	1	Rare on North Island	MH751487
Norhap06	1	Rare on North Island	MH751488
Rathap01	176	Upper North Island (north of the Auckland isthmus); most of the South Island (excluding Southland) and neighboring islands; Big South Cape Island (off Stewart Island); Lord Howe Island; Norfolk Island; Port Jackson, Sydney	KR559034
Rathap02	141	Most of the North Island (south of the Auckland isthmus); southern-most South Island (Southland) and neighboring islands; Ponui Island, Kawau Island, Kaikoura Island and neighbours, and Rakitu Island (all Hauraki Gulf); Macquarie Island; Chatham Island.	KR559035
Rathap03	8	Township of Bluff on South Island	KR559036
Rathap04	27	Great Barrier Island and Kaikoura Island and neighbours	MH751489
Rathap05	1	Rare on Kaikoura Island	MH751490
Rathap06	3	Rare on Kaikoura Island	MH751491
Rathap07	57	Stewart Island and neighboring islands	KR559037
Rathap08	0	Known from museum samples from Stewart Island (Robins et al., 2016)	KR559038
Rathap09	1	Rare on Stewart Island	KR559039
Rathap10	2	Lord Howe Island	MH751492
Rathap11	1	Rare on North Island	MH751493
Rathap12	1	Rare on North Island	MH751494
Rathap13	1	Rare on South Island	MH751495
Rathap14	1	Rare on South Island	MH751496
Rathap15	1	Rare on South Island	MH751497
Rathap16	1	Rare on South Island	MH751498
Rathap17	3	Rangitoto Island	MH751499

Rattus norvegicus

A total of 130 samples of *R. norvegicus*, from 24 islands, were included in our study. Notable absences included Kapiti and Mayor Islands (eradicated and no samples located). The aligned sequences, 545 nucleotides long, had differences in the base composition at 12 positions, and six haplotypes were found (**Table 2**). The relationships among the haplotypes are shown in **Figure 1**, and their distribution across New Zealand and surrounding islands in **Figure 2**.

Two geographically partitioned haplotypes were predominant. Norhap01 was found throughout the North Island and neighboring islands; Raoul Island; Chatham Island; and Campbell Island. The substantially different Norhap02 was found throughout the South Island and neighboring islands. Both Norhap01 and Norhap02 were found on Stewart Island, but Norhap01 was restricted to the port town of Oban. Forming a haplogroup with Norhap01, single instances of Norhap04, Norhap05 and Norhap06, singleton variations of Norhap01, were also found scattered throughout the North Island. The distinct haplotype Norhap03, intermediate between Norhap1 and Norhap02, was found in the port town of Bluff.

Rattus rattus

A total of 425 samples of *R. rattus*, from 31 islands, were included in our study. The only absence of note was Arapawa Island (extant but no samples located). The aligned sequences, 545 nucleotides long, had differences in the base composition at 17 positions, and 17 haplotypes were found (**Table 3**). The relationships among the haplotypes are shown in **Figure 3** and their distribution across New Zealand and surrounding islands in **Figures 4**–**6**.

Two geographically partitioned haplotypes were predominant. Rathap01 was found in the upper North Island (north of the Auckland isthmus); most of the South Island (excluding Southland) and neighboring islands; as well as Big South Cape Island (off Stewart Island); Lord Howe Island; Norfolk Island; and Port Jackson, Sydney. The closely related Rathap02 was found in most of the North Island (south of the Auckland isthmus); southern-most South Island (Southland) and neighboring islands; as well as Ponui Island, Kawau Island, Kaikoura Island and neighbours, and Rakitu Island (all Hauraki Gulf, **Figure 5**); Macquarie Island; and Chatham Island. Two other haplotypes were common. Rathap04 was found in Great Barrier Island and Kaikoura Island and neighbours,

TABLE 2 | Differences in the base composition of *R. norvegicus* haplotypes from across the New Zealand archipelago.

Position	15507	15549	15568	15569	15579	15588	15589	15590	15609	15616	15638	15810
Norhap01	T	T	G	A	A	T	A	T	T	G	T	T
Norhap02	C	.	A	C	G	C	.	C	C	.	.	C
Norhap03	C	.	A	.	G	.	G	.	.	.	.	C
Norhap04	.	.	.	.	.	.	.	.	.	A	.	.
Norhap05	.	C	.	.	.	.	.	.	.	.	.	.
Norhap06	.	.	.	.	.	.	.	.	.	.	C	.

Base positions are numbered relative to the mitochondrial reference genome for R. norvegicus (GenBank NC_001665.2).

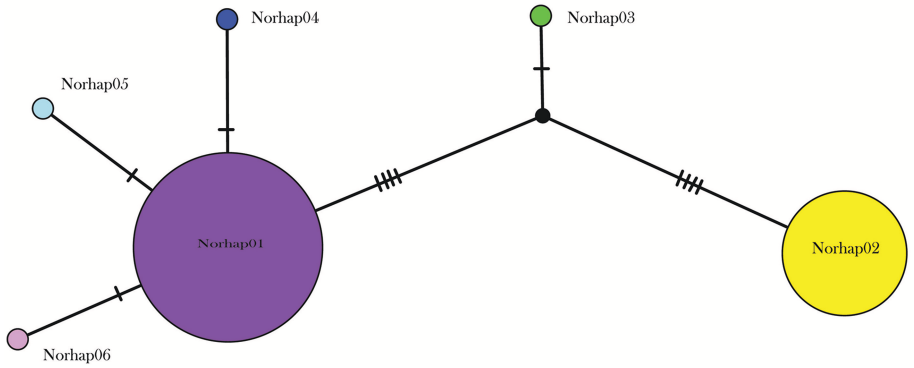

FIGURE 1 | Median joining haplotype network for 130 *R. norvegicus* individuals from across the New Zealand archipelago.

and Rathap07 was found in Stewart Island and neighboring islands (**Figure 6**).

Forming a haplogroup with Rathap01, a few instances of Rathap17 and Rathap03, singleton variations of Rathap01, were found on Rangitoto Island and in the port town of Bluff. Single instances of Rathap13, Rathap14, Rathap15, and Rathap16, singleton variations of Rathap01, were also found scattered throughout the South Island. Rathap10, also a singleton variation of Rathap01, was found on Lord Howe Island. Forming a haplogroup with Rathap02, single instances of Rathap11 and Rathap12, singleton variations of Rathap02, were also found scattered throughout the North Island. Forming a haplogroup with Rathap04, a few instances of Rathap05 and Rathap06, singleton variations of Rathap04, were also found on Kaikoura Island. Forming a haplogroup with Rathap07, a single instance of Rathap09, a singleton variation of Rathap07, was also found on Stewart Island. We only had coverage of the area of the genome distinguishing RatHap09 from RatHap07 for 25% of samples from Stewart Island (the only location where either haplotype occurred). However, we had coverage of this area of the genome for 60% of samples in total, and did not find any further mutations. Nonetheless, RatHap09 might be under-represented in our results.

DISCUSSION

The phylogeographies of both rat species introduced by Europeans exhibit marked geographic partitioning between distinct haplogroups, suggesting that more than one introduction of each species took place. Multiple independent introductions have also been inferred from mitochondrial DNA haplotypes of *R. exulans* and *M. musculus* in New Zealand (Matisoo-Smith et al., 1998; King et al., 2016).

From known historical records *R. norvegicus* was first observed in the North Island in 1772 and was widespread by the 1830s, while in the South Island the first observations were in the 1850s (Innes, 2005a), and was first observed in Stewart Island in the 1870s (Thomson, 1922). These records are in agreement with the two haplogroups identified, which are suggestive of at least two independent introductions. In keeping with the earlier North Island introduction date, single base-pair mutations from the dominant haplogroup were only detected in the North Island. Similar within-archipelago independent introductions of *R. norvegicus* have also been found in the Falklands Islands (Hingston et al., 2016). Large gaps in our coverage of the South Island for *R. norvegicus* correspond with the extent of alpine and beech forest distribution in New Zealand (Wardle, 1984), from where *R. norvegicus* is seemingly absent.

From known historical records *R. rattus* was only widespread in the North Island and Great Barrier Island after 1860, in the South Island after 1890, and was first observed on Stewart Island in 1911 (Atkinson, 1973). These records are in agreement with the four geographically partitioned haplogroups identified, which are suggestive of at least

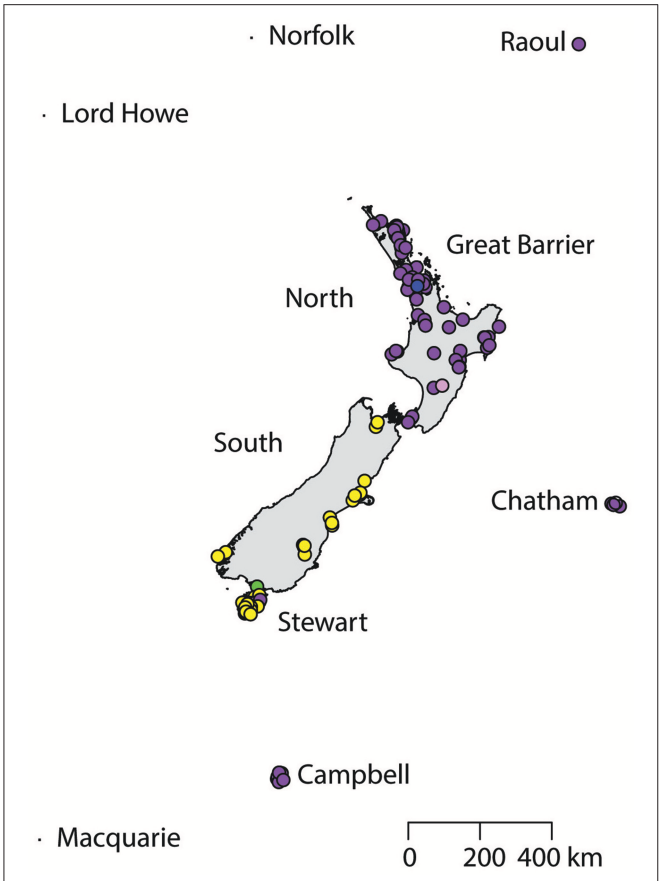

FIGURE 2 | The distribution of *R. norvegicus* haplotypes across New Zealand and surrounding islands.

four independent introductions across the North Island, South Island, Great Barrier Island, and Stewart Island. In keeping with the similar introduction dates, single base-pair mutations from the dominant haplogroups were detected on all four islands. Together this evidence suggests invasion and spread were rapid across islands, and therefore a limited role for bridgehead effects from subsequent invasions (Bertelsmeier et al., 2018). Instead, incumbent advantage appears to have inhibited over-invasion by conspecifics (Waters et al., 2013).

For both species our findings most likely represent a minimum number of introductions, as additional introductions of an already-extant haplotype may also have taken place, perhaps explaining observations such as the dispersed geographic distributions of Rathap01 and Rathap02 across the North Island and South Island. Rare pre- or post-introduction mutations of single base pairs also cannot be unequivocally distinguished from additional introductions. Although there is good national coverage, sampling intensity was generally low within sites across the North Island and South Island. Thus, with rare haplotypes being detected, the results presented here should be interpreted cautiously (Muirhead et al., 2008).

Many of the haplotypes found in this study have been reported in other studies worldwide, but global representation is too patchy to support conclusive inference on historical invasion pathways to New Zealand. For *R. norvegicus*, Norhap01 was the most common haplotype found in modern samples from England (Haniza et al., 2015), while Norhap02 has only been reported in China (Liu et al., 2012). For *R. rattus*, Rathap01 has been found in modern relict island populations in England (Hingston et al., 2005) and across the Pacific Islands from New Guinea to French Polynesia (Robins et al., 2007). Closely related haplotypes to Rathap01 and Rathap02 have been found throughout the Mediterranean basin (Colangelo et al., 2015). Rathap04 has not been reported anywhere previously, while Rathap07 has been reported in New South Wales, Australia (Rowe et al., 2011). These links reaffirm the correspondence of *R. rattus* and *R. norvegicus* with European movements throughout the wider Pacific. The Asian link with Norhap02 might indicate a non-European origin for *R. norvegicus* in the South Island of New Zealand, as has also been inferred for introduced mice in the southern South Island (King et al., 2016).

Our study also detected evidence of ongoing contemporary movement of both rat species within New Zealand, particularly where we had comprehensive sampling on Great Barrier and Stewart Islands. A subset of data from this study has already been used to determine the putative distant origin of the *R. rattus* which invaded Big South Cape Island, off Stewart Island, in the 1960s (Robins et al., 2016). Stewart Island itself was probably colonized first by the South Island haplotype of *R. norvegicus*, and then more recently by the North Island haplotype restricted to the port town of Oban. Similarly, unique haplotypes were found for both *R. norvegicus* and *R. rattus* in the southern port town of Bluff, and had seemingly not spread far beyond this locality. Kaikoura Island and its neighboring islands (Nelson Island and Motuhaku Island), off the western coast of Great Barrier Island, had an unusual number of *R. rattus* haplotypes, with five haplotypes identified from 29 samples comprising a mix of the North Island and Great Barrier Island haplotypes, possibly indicative of recent long-distance transportation by boat. Invasive rats demonstrate a strong incumbent advantage both between and within species (Russell et al., 2014), so localized occurrence of a haplotype may indicate that this haplotype arrived subsequent to the initial invasion. This is especially suggestive for *R. norvegicus,* where the Bluff haplotype differs from all other sampled haplotypes by several base pairs, so is not a plausible *in-situ* mutation. Together, these results are suggestive that long-distance rat movements occurred at least well into the twentieth century.

The documented proportion of *R. rattus* pelage phenotypes in a region also appears to correspond to our haplotype distributions (Innes, 2005b). For example, higher proportions of the melanistic "rattus" (black) pelage occur in the South Island and upper North Island, corresponding with haplotype Rathap01, but are almost absent from Stewart Island, corresponding with Rathap07. The inheritance of pelage color is through known genetic markers unrelated to mtDNA haplotype (Kambe et al., 2011), but a geographical

TABLE 3 | Differences in the base composition of *R. rattus* haplotypes from across the New Zealand archipelago.

Position	15469	15487	15489	15513	15543	15563	15572	15581	15604	15619	15653	15716	15757	15760	15761	15808	15822
Rathap01	T	T	T	C	C	T	A	T	A	G	C	T	C	A	G	C	T
Rathap02	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
Rathap03	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
Rathap04	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Rathap05	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.
Rathap06	C	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.
Rathap07	.	.	.	.	T	.	.	.	.	.	.	.	.	G	.	.	.
Rathap08	.	.	.	.	T	.	.	.	.	.	T	.	.	G	.	.	.
Rathap09	.	.	.	.	T	.	.	.	.	.	.	.	.	G	.	.	C
Rathap10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
Rathap11	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
Rathap12	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.
Rathap13	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rathap14	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
Rathap15	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
Rathap16	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
Rathap17	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.

Base positions are numbered relative to the mitochondrial reference genome for R. rattus (GenBank NC_012374).

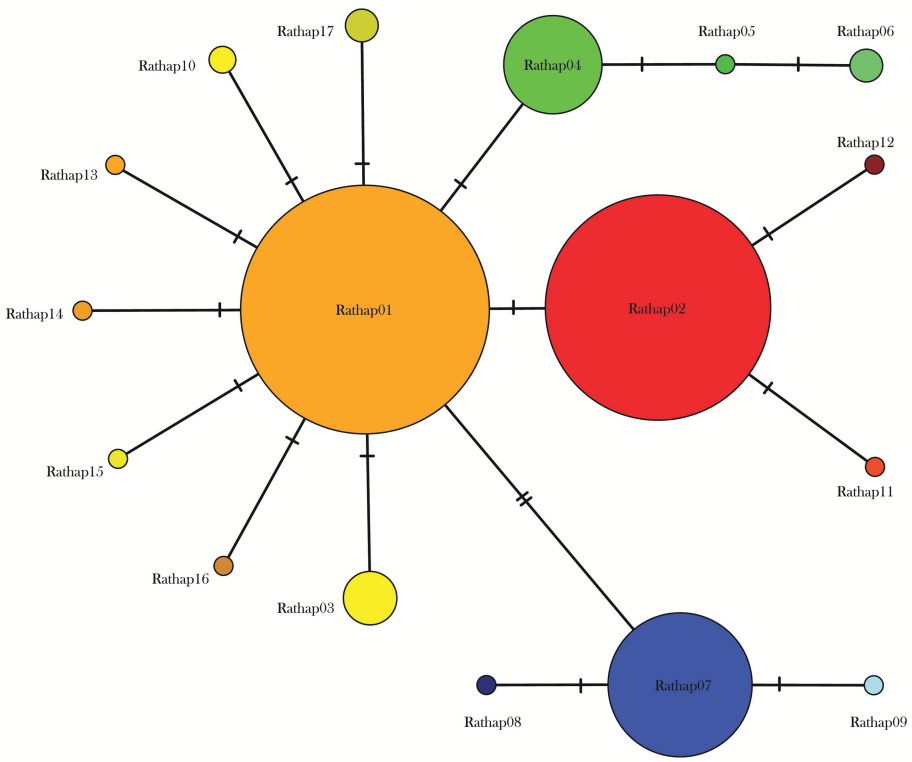

FIGURE 3 | Median joining haplotype network for 425 *R. rattus* individuals from across the New Zealand archipelago.

association between pelage color and haplogroup may arise due to the genetic makeup of different founding populations. Similarly, the rare occurrence in *R. rattus* of the Tyr25Phe mutation in VKorc1, which is associated with anti-coagulant resistance, also corresponds with the distribution of haplotype Rathap02 (Cowan et al., 2017).

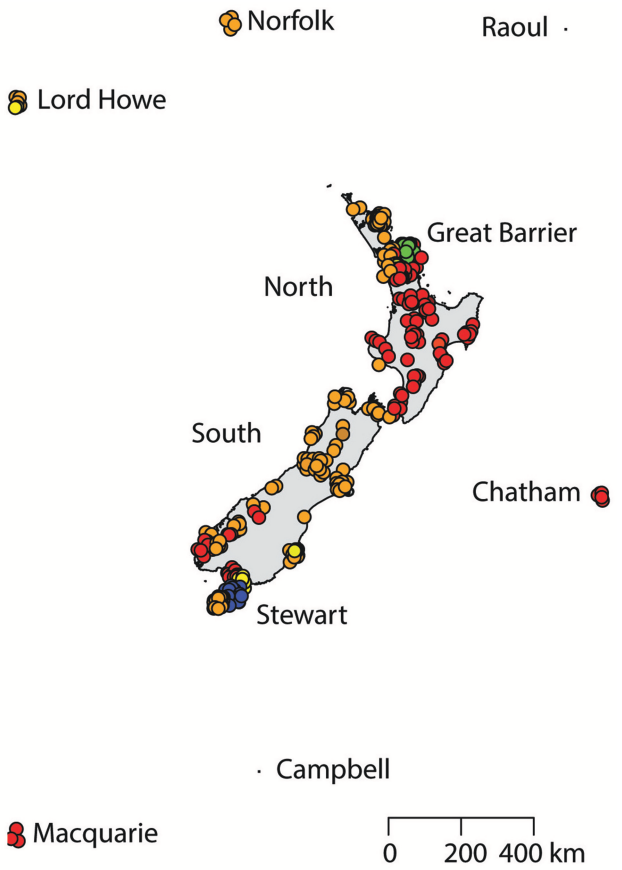

FIGURE 4 | The distribution of *R. rattus* haplotypes across New Zealand and surrounding islands.

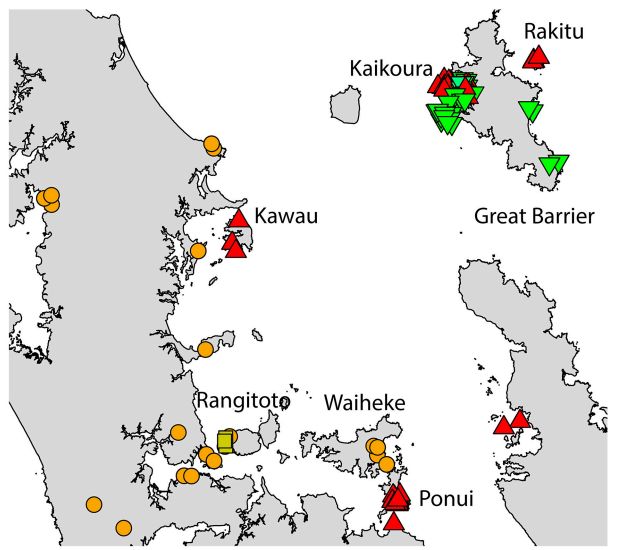

FIGURE 5 | The distribution of *R. rattus* haplotypes across the Hauraki Gulf, Auckland. ● = RatHap01, ▲ = RatHap02, ▼ = RatHap04, RatHap05 & RatHap06, □ = RatHap17.

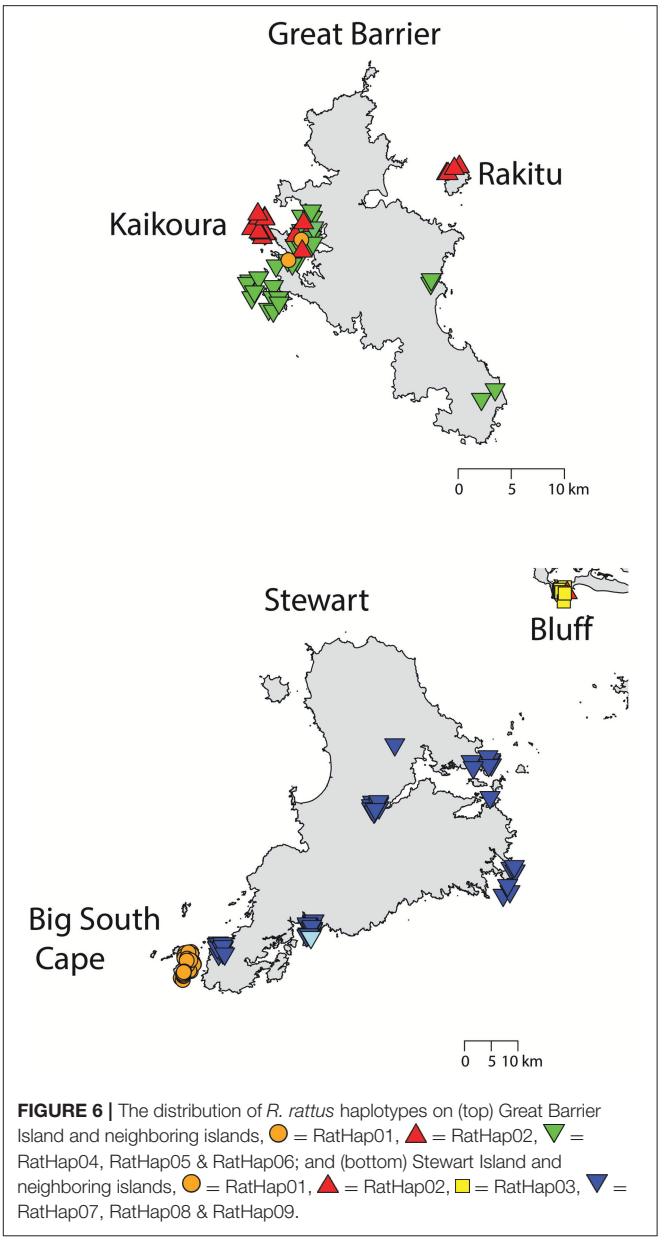

FIGURE 6 | The distribution of *R. rattus* haplotypes on (top) Great Barrier Island and neighboring islands, ● = RatHap01, ▲ = RatHap02, ▼ = RatHap04, RatHap05 & RatHap06; and (bottom) Stewart Island and neighboring islands, ● = RatHap01, ▲ = RatHap02, □ = RatHap03, ▼ = RatHap07, RatHap08 & RatHap09.

Advances in molecular biology since this work was undertaken already allow higher resolution characterisation of individual genetic variability. Single nucleotide polymorphisms (SNPs) in particular facilitate deeper insight into the population genetics of invasive rats (e.g., Puckett et al., 2016). Results at a higher resolution from future genetic studies of invasive rats in New Zealand may help further distinguish independent introductions, and better facilitate characterisation of invasion spread across New Zealand. Such studies may be facilitated by the availability of high-throughput tools such as SNP chips developed for medical research on laboratory rats, once ascertainment bias has been accounted for. Ultimately, the availability of low-cost genomes for individual invasive rats will usher in the era of population genomics (Aitman et al., 2008).

AUTHOR CONTRIBUTIONS

JCR and RF conceived the research. JCR and RF oversaw sample acquisition. JHR performed laboratory analyses. JHR and JCR undertook analysis. JCR wrote the manuscript with input from RF.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00048/full#supplementary-material

Supplementary Table 1 | Samples including species, island, haplotype, and coordinates. *indicates museum specimen.

REFERENCES

Abdelkrim, J., Byrom, A. E., and Gemmell, N. J. (2010). Fine-scale genetic structure of mainland invasive *Rattus rattus* populations: implications for restoration of forested conservation areas in New Zealand. *Conserv. Genet.* 11, 1953–1964. doi: 10.1007/s10592-010-0085-9

Aitman, T. J., Critser, J. K., Cuppen, E., Dominiczak, A., Fernandez-Suarez, X. M., Flint, J., et al. (2008). Progress and prospects in rat genetics: a community view. *Nat. Genet.* 40, 516–522. doi: 10.1038/ng.147

Aplin, K. P., Suzuki, H., Chinen, A. A., Chesser, R. T., Ten Have, J., Donnellan, S. C., et al. (2011). Multiple geographic origins of commensalism and complex dispersal history of black rats. *PLoS ONE.* 6:e26357. doi: 10.1371/journal.pone.0026357

Atkinson, I. A. E. (1973). Spread of the ship rat (*Rattus r. rattus L.)* in New Zealand. *J. R. Soc. N. Z.* 3, 457–472. doi: 10.1080/03036758.1973.10421869

Bandelt, H. J., Forster, P., and Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48. doi: 10.1093/oxfordjournals.molbev.a026036

Bassett, I. E., Cook, J., Buchanan, F., and Russell, J. C. (2016). Treasure islands: biosecurity in the Hauraki Gulf marine park. *N. Z. J. Ecol.* 40, 250–266. doi: 10.20417/nzjecol.40.28

Bastos, A. D., Nair, D., Taylor, P. J., Brettschneider, H., Kirsten, F., Mostert, E., et al. (2011). Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive *Rattus* congeners in South Africa. *BMC. Genet.* 12:26. doi: 10.1186/1471-2156-12-26

Bertelsmeier, C., Ollier, S., Liebhold, A. M., Brockerhoff, E. G., Ward, D., and Keller, L. (2018). Recurrent bridgehead effects accelerate global alien ant spread. *Proc. Natl. Acad. Sci. U.S.A.* 115, 5486–5491. doi: 10.1073/pnas.1801990115

Berthier, K., Garba, M., Leblois, R., Navascués, M., Tatard, C., Gauthier, P., et al. (2016). Black rat invasion of inland Sahel: insights from interviews and population genetics in south-western Niger. *Biol. J. Linn. Soc.* 119, 748–765. doi: 10.1111/bij.12836

Brouat, C., Rahelinirina, S., Loiseau, A., Rahalison, L., Rajerison, M., Laffly, D., et al. (2013). Plague circulation and population genetics of the reservoir *Rattus rattus*: the influence of topographic relief on the distribution of the disease within the Madagascan focus. *PLoS Negl. Trop. Dis.* 7:e2266. doi: 10.1371/journal.pntd.0002266

Brouat, C., Tollenaere, C., Estoup, A., Loiseau, A., Sommer, S., Soanandrasana, R., et al. (2014). Invasion genetics of a human commensal rodent: the black rat *Rattus rattus* in Madagascar. *Mol. Ecol.* 23, 4153–4167. doi: 10.1111/mec.12848

Brown, K., Elliott, G., Innes, J., and Kemp, J. (2015). *Ship Rat, Stoat and Possum Control on Mainland New Zealand. An Overview of Techniques, Successes and Challenges.* Wellington: Department of Conservation.

Clout, M. N., and Russell, J. C. (2006) "The eradication of mammals from New Zealand islands," in *Assessment and Control of Biological Invasion Risks,* eds F. Koike, M. N. Clout, M. Kawamichi, M. De Poorter, K. Iwatsuki (Gland: IUCN; Kyoto: Shoukadoh Book Sellers), 127–141.

Colangelo, P., Abiadh, A., Aloise, G., Amori, G., Capizzi, D., Vasa, E., et al. (2015). Mitochondrial phylogeography of the black rat supports a single invasion of the western Mediterranean basin. *Biol. Invasions* 17, 1859–1868. doi: 10.1007/s10530-015-0842-2

Conroy, C. J., Rowe, K. C., Rowe, K. M., Kamath, P. L., Aplin, K. P., Hui, L., et al. (2013). Cryptic genetic diversity in *Rattus* of the San Francisco Bay region, California. *Biol. Invasions* 15, 741–758. doi: 10.1007/s10530-012-0323-9

Cowan, P. E., Gleeson, D. M., Howitt, R. L., Ramón-Laca, A., Esther, A., and Pelz, H. J. (2017). Vkorc1 sequencing suggests anticoagulant resistance in rats in New Zealand. *Pest Manag. Sci.* 73, 262–266. doi: 10.1002/ps.4304

Fewster, R. M., Miller, S. D., and Ritchie, J. (2011). "DNA profiling - a management tool for rat eradication," in *Island Invasives: Eradication and Management*, eds C. R. Veitch, M. N. Clout, and D. R. Towns (Gland: IUCN), 430–435.

Haniza, M. Z., Adams, S., Jones, E. P., MacNicoll, A., Mallon, E. B., Smith, R. H., et al. (2015). Large-scale structure of brown rat (*Rattus norvegicus*) populations in England: effects on rodenticide resistance. *PeerJ* 3:e1458. doi: 10.7717/peerj.1458

Hingston, M., Goodman, S. M., Ganzhorn, J. U., and Sommer, S. (2005). Reconstruction of the colonization of southern Madagascar by introduced *Rattus rattus*. *J. Biogeogr.* 32, 1549–1559. doi: 10.1111/j.1365-2699.2005.01311.x

Hingston, M., Poncet, S., Passfield, K., Tabak, M. A., Gabriel, S. I., Piertney, S. B., et al. (2016). Phylogeography of *Rattus norvegicus* in the South Atlantic Ocean. *Diversity* 8:32. doi: 10.3390/d8040032

Innes, J. G. (2005a). "Norway rat," in *The Handbook of New Zealand Mammals. 2nd edition*, ed C. M. King, (Melbourne, VIC: Oxford University Press), 174–187.

Innes, J. G. (2005b). "Ship rat," in *The Handbook of New Zealand Mammals. 2nd edition*, ed C. M. King, (Melbourne, VIC: Oxford University Press), 187–203.

Kajdacsi, B., Costa, F., Hyseni, C., Porter, F., Brown, J., Rodrigues, G., et al. (2013). Urban population genetics of slum-dwelling rats (*Rattus norvegicus*) in Salvador, Brazil. *Mol. Ecol.* 22, 5056–5070. doi: 10.1111/mec.12455

Kaleme, P. K., Bates, J. M., Belesi, H. K., Bowie, R. C. K., Gambalemoke, M., Kerbis-Peterhans, J., et al. (2011). Origin and putative colonization routes for invasive rodent taxa in the Democratic Republic of Congo. *Afr. Zool.* 46, 133–145. doi: 10.1080/15627020.2011.11407486

Kambe, Y., Tanikawa, T., Matsumoto, Y., Tomozawa, M., Aplin, K. P., and Suzuki, H. (2011). Origin of agouti-melanistic polymorphism in wild black rats (*Rattus rattus*) inferred from Mc1r gene sequences. *Zool. Sci.* 28, 560–567. doi: 10.2108/zsj.28.560

King, C., Alexander, A., Chubb, T., Cursons, R., MacKay, J., McCormick, H., et al. (2016). What can the geographic distribution of mtDNA haplotypes tell us about the invasion of New Zealand by house mice *Mus musculus*? *Biol. Invasions* 18, 1551–1565. doi: 10.1007/s10530-016-1100-y

Konečný, A., Estoup, A., Duplantier, J. M., Bryja, J., Bâ, K., Galan, M., et al. (2013). Invasion genetics of the introduced black rat (*Rattus rattus*) in Senegal, West Africa. *Mol. Ecol.* 22, 286–300. doi: 10.1111/mec.12112

Lack, J. B., Greene, D. U., Conroy, C. J., Hamilton, M. J., Braun, J. K., Mares, M. A., et al. (2012). Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. *Mol. Ecol.* 21, 3545–3561. doi: 10.1111/j.1365-294X.2012.05620.x

Lack, J. B., Hamilton, M. J., Braun, J. K., Mares, M. A., and Van Den Bussche, R. A. (2013). Comparative phylogeography of invasive *Rattus rattus* and *Rattus norvegicus* in the US reveals distinct colonization histories and dispersal. *Biol. Invasions* 15, 1067–1087. doi: 10.1007/s10530-012-0351-5

Liu, J., Liu, D.-Y., Chen, W., Li, J.-L., Luo, F., Li, Q., et al. (2012). Genetic analysis of hantaviruses and their rodent hosts in central-south China. *Virus Res.* 163, 439–447. doi: 10.1016/j.virusres.2011.11.006

López, M., Foronda, P., Feliu, C., and Hernández, M. (2013). Genetic characterization of black rat (*Rattus rattus*) of the Canary Islands: origin and colonization. *Biol. Invasions* 15, 2367–2372. doi: 10.1007/s10530-013-0466-3

Matisoo-Smith, E., Roberts, R. M., Irwin, G. J., Allen, J. S., Penny, D., and Lambert, D. M. (1998). Patterns of prehistoric human mobility in polynesia indicated by mtDNA from the Pacific rat. *Proc. Natl Acad. Sci. U.S.A.* 95, 15145–15150. doi: 10.1073/pnas.95.25.15145

Muirhead, J. R., Gray, D. K., Kelly, D. W., Ellis, S. M., Heath, D. D., and MacIsaac, H. J. (2008). Identifying the source of species invasions: sampling intensity vs. genetic diversity. *Mol. Ecol.* 17, 1020–1035. doi: 10.1111/j.1365-294X.2008.03669.x

Puckett, E. E., Park, J., Combs, M., Blum, M. J., Bryant, J. E., Caccone, A., et al. (2016). Global population divergence and admixture of the brown rat (*Rattus norvegicus*). *Proc. R. Soc.* 283:20161762. doi: 10.1098/rspb.2016.1762

Richardson, J. L., Burak, M. K., Hernandez, C., Shirvell, J. M., Mariani, C., Carvalho-Pereira, T. S., et al. (2017). Using fine-scale spatial genetics of Norway rats to improve control efforts and reduce leptospirosis risk in urban slum environments. *Evol. Appl.* 10, 323–337. doi: 10.1111/eva.12449

Robins, J. H., Hingston, M., Matisoo-Smith, E., and Ross, H. A. (2007). Identifying *Rattus* species using mitochondrial DNA. *Mol. Ecol. Resour.* 7, 717–729. doi: 10.1111/j.1471-8286.2007.01752.x

Robins, J. H., Miller, S. D., Russell, J. C., Harper, G. A., and Fewster, R. M. (2016). Where did the rats of Big South Cape Island come from? *N. Z. J. Ecol.* 40, 229–234. doi: 10.20417/nzjecol.40.26

Rowe, K. C., Aplin, K. P., Baverstock, P. R., and Moritz, C. (2011). Recent and rapid speciation with limited morphological disparity in the genus *Rattus*. *Syst. Biol.* 60, 188–203. doi: 10.1093/sysbio/syq092

Russell, J. C., and Broome, K. G. (2016). Fifty years of rodent eradications in New Zealand: another decade of advances. *N. Z. J. Ecol.* 40, 197–204. doi: 10.20417/nzjecol.40.22

Russell, J. C., and Clout, M. N. (2004). Modelling the distribution and interaction of introduced rodents on New Zealand offshore islands. *Global Ecol. Biogeograp.* 13, 497–507. doi: 10.1111/j.1466-822X.2004.00124.x

Russell, J. C., Innes, J. G., Brown, P. H., and Byrom, A. E. (2015). Predator-free New Zealand: conservation country. *BioScience* 65, 520–525. doi: 10.1093/biosci/biv012

Russell, J. C., Mackay, J. W., and Abdelkrim, J. (2009). Insular pest control within a metapopulation context. *Biol. Conserv.* 142, 1404–1410. doi: 10.1016/j.biocon.2009.01.032

Russell, J. C., Miller, S. D., Harper, G. A., MacInnes, H. E., Wylie, M. J., and Fewster, R. M. (2010). Survivors or reinvaders? Using genetic assignment to identify invasive pests following eradication. *Biol. Invasions* 12, 1747–1757. doi: 10.1007/s10530-009-9586-1

Russell, J. C., Sataruddin, N. S., and Heard, A. D. (2014). Over-invasion by functionally equivalent invasive species. *Ecology* 95, 2268–2276. doi: 10.1890/13-1672.1

Russell, J. C., Towns, D. R., Anderson, S. H., and Clout, M. N. (2005). Intercepting the first rat ashore. *Nature* 437:1107. doi: 10.1038/4371107a

Searle, J. B., Jamieson, P. M., Gündüz, I., Stevens, M. I., Jones, E. P., Gemmill, C. E., et al. (2009). The diverse origins of New Zealand house mice. *Proc. Biol. Sci.* 276, 209–217. doi: 10.1098/rspb.2008.0959

Song, Y., Lan, Z., and Kohn, M. H. (2014). Mitochondrial DNA phylogeography of the Norway rat. *PLoS ONE.* 9:e88425. doi: 10.1371/journal.pone.0088425

Theuerkauf, J., Kuehn, R., Gula, R., Sztencel-Jabłonka, A., Jourdan, H., Taugamoa, A., et al. (2015). Invasion history affects genetic structure in island rat populations. *J. Zool.* 295, 197–205. doi: 10.1111/jzo.12206

Thomson, G. M. (1922). *The Naturalization of Animals & Plants in New Zealand*. London: Cambridge University Press.

Tollenaere, C., Brouat, C., Duplantier, J. M., Rahalison, L., Rahelinirina, S., Pascal, M., et al. (2010). Phylogeography of the introduced species *Rattus rattus* in the western Indian Ocean, with special emphasis on the colonization history of Madagascar. *J. Biogeogr.* 37, 398–410. doi: 10.1111/j.1365-2699.2009.02228.x

Tollenaere, C., Ivanova, S., Duplantier, J. M., Loiseau, A., Rahalison, L., Rahelinirina, S., et al. (2012). Contrasted patterns of selection on MHC-linked microsatellites in natural populations of the malagasy plague reservoir. *PLoS ONE.* 7:e32814. doi: 10.1371/journal.pone.0032814

Varudkar, A., and Ramakrishnan, U. (2015). Commensalism facilitates gene flow in mountains: a comparison between two *Rattus* species. *Heredity* 115, 253–261. doi: 10.1038/hdy.2015.34

Veale, A. J., Russell, J. C., and King, C. M. (2018). The genomic ancestry, landscape genetics and invasion history of introduced mice in New Zealand. *R. Soc. Open Sci.* 5:170879. doi: 10.1098/rsos.170879

Wardle, J. (1984). *The New Zealand Beeches: Ecology, Utilisation and Management*. Wellington: New Zealand Forest Service.

Waters, J. M., Fraser, C. I., and Hewitt, G. M. (2013). Founder takes all: density-dependent processes structure biodiversity. *Trends Ecol. Evol.* 28, 78–85. doi: 10.1016/j.tree.2012.08.024

Wilmshurst, J. M., Anderson, A. J., Higham, T. F., and Worthy, T. H. (2008). Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *Proc. Natl. Acad. Sci. U.S.A.* 105, 7676–7680. doi: 10.1073/pnas.0801507105

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Evidence of Influenza A in Wild Norway Rats (*Rattus norvegicus*) in Boston, Massachusetts

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Influenza A virus (IAV) is known to circulate among human and animal reservoirs, yet there are few studies that address the potential for urban rodents to carry and shed IAV. Rodents are often used as influenza models in the lab, but the few field studies that have looked for evidence of IAV in rodents have done so primarily in rural areas following outbreaks of IAV in poultry. This study sought to assess the prevalence of IAV recovered from wild Norway rats in a dense urban location (Boston). To do this, we sampled the oronasal cavity, paws, and lungs of Norway rats trapped by the City of Boston's Inspectional Services from December 2016 to September 2018. All samples were screened by real-time, reverse transcriptase PCR targeting the conserved IAV matrix segment. A total of 163 rats were trapped, 18 of which (11.04%) were RT-PCR positive for IAV in either oronasal swabs (9), paw swabs (9), both (2), or lung homogenates (2). A generalized linear model indicated that month and geographic location were correlated with IAV-positive PCR status of rats. A seasonal trend in IAV-PCR status was observed with the highest prevalence occurring in the winter months (December–January) followed by a decline over the course of the year, reaching its lowest prevalence in September. Sex and weight of rats were not significantly associated with IAV-PCR status, suggesting that rodent demography is not a primary driver of infection. This pilot study provides evidence of the need to further investigate the role that wild rats may play as reservoirs or mechanical vectors for IAV circulation in urban environments across seasons.

Keywords: wildlife disease, urban rodents, Orthomyxoviridae, novel host, influenza

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INTRODUCTION

Influenza A virus (IAV) is a single-stranded, negative-sense, RNA virus with a segmented genome that belongs to the family Orthomyxoviridae. It is a virus that has impacted human populations since the nineteenth century and likely earlier (Barry, 2004; Taubenberger et al., 2007). Pandemic viral outbreaks, the worst of which was the Spanish Flu of 1918, can kill millions of otherwise healthy people worldwide, while seasonal influenza kills thousands of people every year and causes billions of dollars in loss of productivity (Molinari et al., 2007). Influenza has a large host range, including domesticated and wild animals. In 2015, an outbreak of highly pathogenic avian influenza (HPAI) cost the U.S. poultry industry one billion dollars and resulted in the culling of 50 million

turkeys and chickens (McKenna, 2015). While such HPAI outbreaks have required extreme control measures as a response, the threat of outbreaks has been continuous in the past 20 years. Aside from strains circulating in poultry (H5NX, H7N9, H9N2), infection with endemically-circulating strains of H3 is commonplace in horses and dogs (Parrish et al., 2015). Persistent IAV infection and circulation amongst swine primarily causes impaired growth and weight loss leading to economic losses for producers (Kothalawala et al., 2006).

Influenza infections have also been documented in numerous species of wildlife, with a growing interest in wild animals that overlap with human settlement and agriculture. Marine mammals, including seals, are known to be infected with both influenza A and B strains (Hinshaw et al., 1984; Puryear et al., 2016). Outbreaks occur periodically in seals, creating opportunities for exposure between seals, humans, and other wildlife that overlap along densely populated coastal margins (Runstadler et al., 2013). It is thought that wild birds, particularly migrating water birds belonging to the orders Anseriformes and Charadriiformes, are the reservoir host of IAV (Webster et al., 1992), and contact with waterfowl is a known risk factor for HPAI outbreaks in poultry (Shortridge et al., 2000). The increasing interface between wild and domestic birds, owing to the conversion of natural wetlands to agriculture, presents challenges for controlling the spread of IAV both in Eurasia and North America (Hill and Runstadler, 2016). Aside from seals and birds, however, relatively few studies have investigated the presence of IAV circulating amongst other wildlife, particularly species such as rodents that come into close contact with humans living in cities.

Wild, urban rodents are a ubiquitous but understudied species that may contribute to the epidemiology of influenza in urban environments. Climate change and milder winters across temperate regions contribute to growing urban rat populations (Atkin and Keizer, 2017). Rats thrive in the built environment allowing for frequent contact with humans and wild birds—both established as important hosts for influenza. Rats also have frequent contact with cats, which are known to transmit influenza to humans, albeit as an influenza host of minor importance (Belser et al., 2017). In addition, rodents harbor zoonotic pathogens like hantaviruses, *Leptospira* sp., arenaviruses, and others (Himsworth et al., 2014). In Vancouver[1], antibiotic-resistant *E. coli* were found in roughly 5% of black and Norway rats, consistent with studies in German cities (Himsworth et al., 2015). In New York City, rats were found to carry *Leptospira* sp., *Bartonella* sp., Seoul virus, gastroenteritis-causing bacteria, as well as a number of previously uncharacterized viruses (Firth et al., 2014). Yet few studies have ever looked for influenza specifically amongst urban wild rodents. In Egypt and Hong Kong, evidence of IAV infection in wild rats and mice has been documented, albeit at a low frequency (Shortridge et al., 2000; Shriner et al., 2012; El-Sayed et al., 2013). In the US, those that have looked for IAV have typically been in the wake of outbreaks in rural settings such as poultry barns (Nettles et al., 1985; Shriner et al., 2012; Grear et al., 2016); however, rodents sampled in these studies were negative for IAV (Nettles et al., 1985; Grear et al., 2016).

The city of Boston, Massachusetts, located in the northeast of the United States is an ideal urban setting to study influenza in wild rodents, as Boston was recently identified as having the second highest level of rodent infestation among large cities as reported by the U.S. Census Bureau; American Housing Survey (2015). To identify the role of wild urban rodents in influenza ecology in the context of a city with significant human and rodent cohabitation, we evaluated the prevalence of IAV carriage among trapped wild rats in Boston over a 2-year period. To our knowledge, this is the first study to consider IAV among wild rodents in a major U.S. city.

MATERIALS AND METHODS

This study was cross-sectional in design and aimed to recover rats from varying microhabitats (parks, alleys, etc.) across the City of Boston from December 2016 to September 2018 (**Figures 1A,B**). Individually bagged Norway rat carcasses were provided by the City of Boston's Inspectional Services. Rodents were collected within 6 h of trapping and were transported on ice to the lab for immediate processing.

Necropsies were conducted aseptically under a laminar flow hood in a BSL-2 laboratory. Rats were sexed and weighed, and swabs of the oronasal cavities and paw pads were obtained using polyester swabs (Puritan, Maine, USA). Oronasal swabs were collected by swabbing the external nares followed by opening the mouth and inserting the swab at the back of the throat at the junction of the oropharynx and nasopharynx. Swabs were immediately placed in viral transport media (VTM: Remel, CA) and frozen at −80°C prior to screening for the viral RNA (Puryear et al., 2016). Lungs were then harvested aseptically, cryofrozen without media or in viral transport media, and stored at −80°C prior to processing.

Lung samples were homogenized for detection of viral RNA. An ∼20 mg piece of lung tissue from each animal was placed into a prefilled 2 ml disruption tube with 2.8 mm stainless steel grinding balls (OPS Diagnostics, NJ) and 350 μl of AE Buffer (Qiagen, Germany) and 350 μl milliQ water. The tube then underwent bead-beating for 1.5 min in a cold room (4°C) prior to further processing.

Viral RNA was extracted from 50 μl of the swab samples in VTM and from lung homogenate samples using the Omega Mag-Bind Viral DNA/RNA kit (Omega Bio-Tek, Norcross, GA, USA) and a Kingfisher Magnetic Particle Processor (Thermo Scientific, Waltham, MA, USA). RNA was screened using qScript XLT One-Step RT-qPCR ToughMix (Quanta Biosciences, Gaithersburg, MD, USA) and analyzed for fluorescence on an ABI 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) for a conserved IAV matrix gene segment (M) target, as previously described (Spackman et al., 2002). VTM was used for negative controls in both extraction and PCR steps. Influenza A/Puerto Rico/8/1934 was used as a positive control for the extraction step and extracted RNA from PR8 strain IAV served as a positive

[1]Detailed necropsy and tissue collection procedure. http://www.vancouverratproject.com/vancouver_rat_project/results Accessed January 21, 2017.

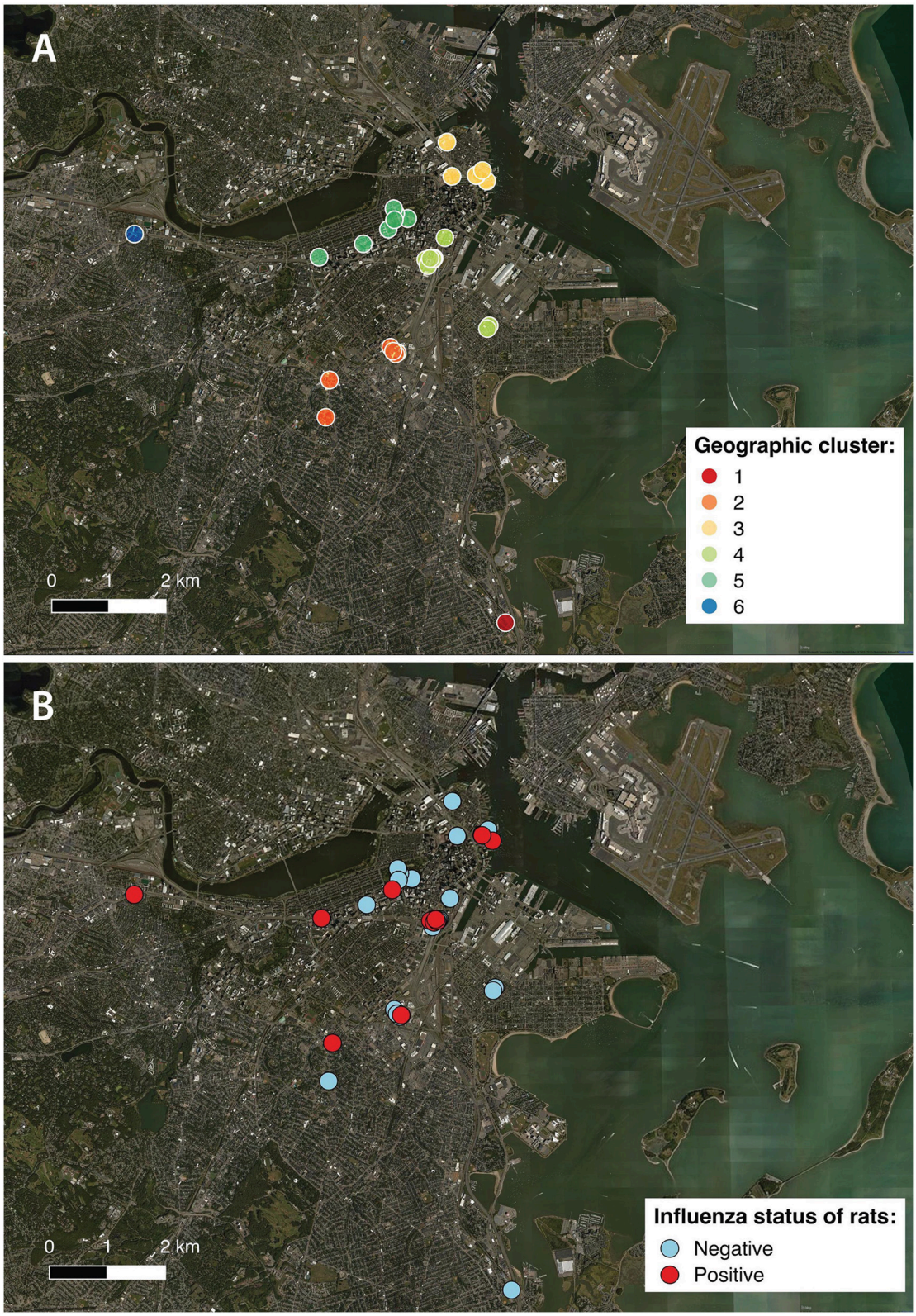

FIGURE 1 | Locations of individual sampling sites within the City of Boston, color coded according to **(A)** the 6 geographical clusters ("geoclusters") identified with k-means clustering, a process that grouped rat sampling sites according to geographic proximity based on latitude and longitude; **(B)** influenza A virus status of rats based on molecular screening (positive or negative).

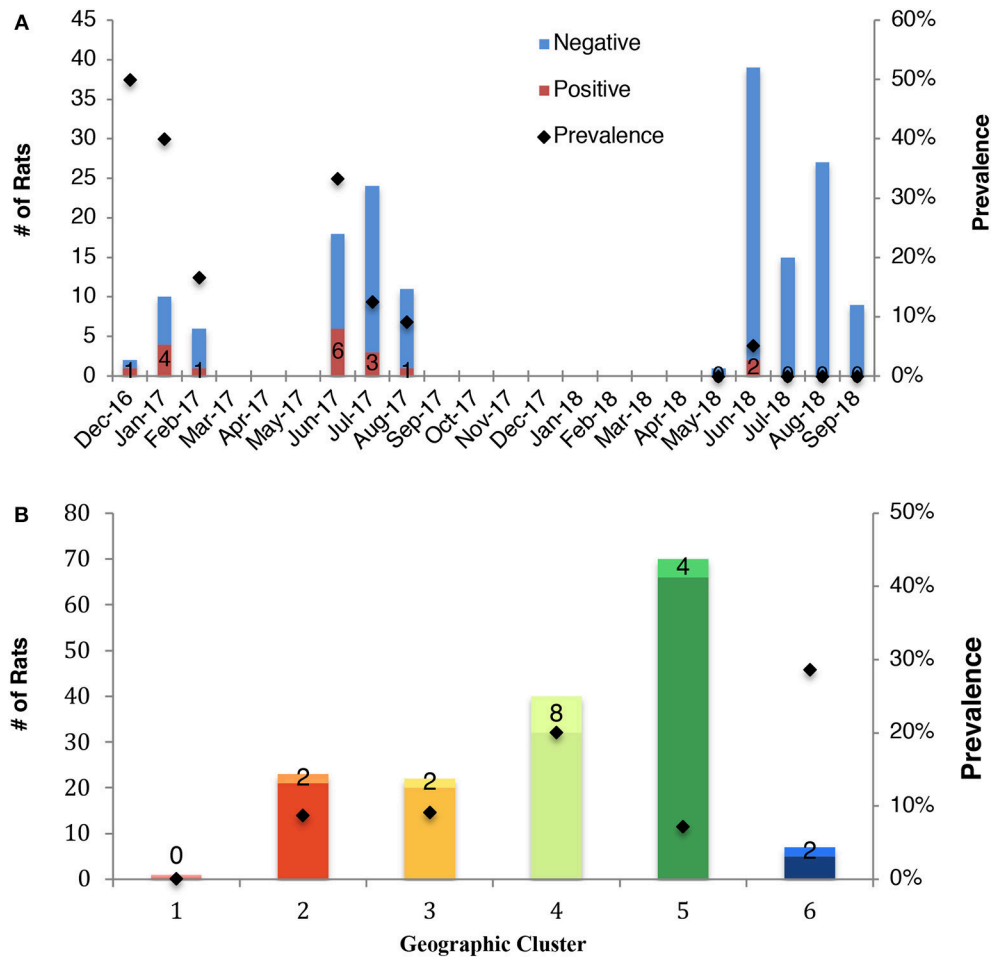

FIGURE 2 | Prevalence of influenza A virus in rats according to **(A)** month of sampling (all months, including those eliminated from statistical analysis are shown), and **(B)** geocluster within the City of Boston. The total sampling effort is shown by the vertical bars. Prevalence is indicated by black marker and the number of positive rats is reported.

control for the PCR step. Samples producing cycle threshold (Ct) values ≤45 were considered positive for IAV RNA. This high Ct cut-off was deemed necessary given the trace amounts of virus associated with wild reservoir species and the potential for inhibitors from the raw sample to reduce detection of virus during PCR.

Positive samples were inoculated into the allantoic cavity of 10-day-old embryonated chicken eggs (ECEs) (Charles River, CT, USA), and incubated at 37°C for 72 h. RNA was extracted from 50 µl of amnio-allantoic fluid (AAF) and screened for the IAV M gene as described above. Whole genome sequencing was attempted on RNA from IAV positive AAF (Ct ≤ 45) and RNA from positive raw samples with a Ct ≤ 37 at the J. Craig Venter Institute in Rockville, MD, as previously described (Nelson et al., 2007). Repeated passage of the positive AAFs was not successful in boosting the viral concentration, reflected by RT-PCR Ct values on passage AAF (data not shown).

Data was analyzed using JMP (JMP®, Version 14.0 SAS Institute Inc., Cary, NC, 1989–2007). Descriptive statistics were used to summarize influenza PCR status (prevalence, 95% confidence intervals) stratified by demographic variables (sex and age).

To group sampling locations that were in geographic proximity, K-mean clustering was performed using latitude and longitude. Data were partitioned from 1 to 20 clusters using an iterative fitting process and the cubic clustering criterion was used to determine the optimal number of partitions or "geoclusters" for the study. K-means clustering indicated that 6 independent geoclusters (**Figure 1A**) was the optimal number of partitions for characterizing the distribution of sampling locations. Mapping of the sampling locations was performed using QGIS 2.18 (qgis.org) and color coded according to geocluster (**Figure 1A**) and influenza infection status (**Figure 1B**).

A generalized linear model (GLM) was used to assess associations between sex (categorical: male/female), weight (continuous: grams), sampling month (ordinal: Jan, Feb, May, June, Jul, Aug, Sep, Dec), and geocluster (categorical: 1–6)

with influenza status expressed as a dichotomous result (i.e., positive/negative). Categories of variables with fewer than 5 data points were excluded from analysis. For instance, month (May, Dec), geocluster 1, and rats of unknown age or sex were all excluded prior to performing GLMs. An information-theoretic approach (Akaike's Information Criterion [AIC]) was used to compare IAV status of rats under different *a priori* defined models relevant to the epidemiology of influenza transmission in wild animals.

To assess whether different specimen types (oronasal, paw, lung) had significantly different Ct values, data were analyzed using one-way ANOVA.

RESULTS

A total of 163 Norway rats were trapped over the course of this study. Eighty-three were female and 72 were male. The mean weight was 164.3 g and ranged from 25 to 525 g. Five rats were neither sexed nor weighed and three rats were weighed but not sexed, and the data was classified as missing; none of these rats were RT-PCR-positive for influenza. An exact binomial test showed that neither males nor females ($p = 0.42$) were trapped at a frequency greater than would be expected by chance alone.

Eighteen of 163 rats, 11.04% (±4.81% 95CI), had swabs or lung samples that were RT-PCR positive for IAV. Nine of 161 had positive oronasal swabs (5.59 ± 3.55% 95CI), and 9 of 161 had positive paw swabs (5.59 ± 3.55% 95CI). Two rats were positive in both oronasal and paw swabs (**Table 1**). Therefore, the recovery of viral RNA from oronasal and paw swabs was equivalent. Only two of 108, 1.85% (±2.54% 95CI), rat lung homogenates were RT-PCR-positive for IAV. Neither individual had positive oronasal or paw swabs (**Table 1**).

The mean Ct value for positive samples was 36.55 (range: 34.36–42.69, STD = 1.78). The mean oronasal swab Ct value was 36.22 (STD = 0.61). The mean paw swab Ct value was 37.19 (STD = 2.43), and the mean lung Ct value was 35.14 (STD = 1.11). Mean Ct values were not significantly different between the three sample types ($p = 0.27$). All Ct values were higher than positive controls, indicating low concentrations of virus in the samples or degradation of the original sample (**Table 1**).

Despite our efforts, we were only able to successfully culture one sample recovered from AAF (**Table 1**). This sample had a Ct value of 41.61 (raw Ct value of 35.21), but sequencing was unsuccessful. None of the raw samples with positive results from initial screening could be successfully sequenced, which precluded identification of the strain or subtype.

Analysis of demographic, morphometric, and spatiotemporal data of rats using GLMs indicated that a model based on month and geocluster provided the best fit for explaining the IAV-PCR status of rats (**Table 2**). Month ($df = 5$, $p = 0.002$) and geocluster ($df = 4$, $p = 0.005$) accounted for 0.782 and 0.173 of the main effect, respectively. The second top-ranked model included month, geocluster, and weight; however, examination of the contribution of weight to the model indicated only a weak effect (main effect = 0.001). Therefore, we determined that the top ranked model was the most parsimonious fit for the data. Weight ($df = 1$, $p = 1.000$) and sex ($df = 1$, $p = 1.000$) of rats were also assessed as model effects but contribute only weakly to explaining the variation in the IAV-PCR status of rats.

The effect of month on IAV status of rats indicated a seasonal signature of IAV circulation. The prevalence of IAV in rats was highest during December and January, followed by a decline over the course of the year, reaching its lowest prevalence in September (**Figure 2A**). The temporal changes in prevalence over the course of the study is presented (**Table S1**) as well as the associated odds ratios (**Table S2**).

A large variation in prevalence was observed between geoclusters within the City of Boston, suggesting that incidence of IAV is spatially patchy (**Figure 2B**). Boston Public Garden and the surrounding area (geocluster 5) were underrepresented for IAV RT-PCR positive rats (**Figures 1A**, **2B**), whereas the neighborhoods of Brighton (geocluster 6) and Chinatown/South Boston (geocluster 4) had a higher incidence of IAV RT-PCR positive rats (**Figure 2B**). Therefore, spatiotemporal factors appear to be more important in determining IAV prevalence, relative to sex and weight of rats.

DISCUSSION

The finding of IAV nucleic acid in urban Norway rats is of public health significance given the close physical proximity between humans and rats in urban environments. Rats are found in alleys, parks, subway systems, and even homes. Our study is among the first to provide evidence that rodents may play a role in the ecology of IAV in dense, urban environments.

The majority of previous studies of IAV in rodents have considered rats in rural areas following outbreaks in poultry or gamebirds and reported zero prevalence (Nettles et al., 1985; Shriner et al., 2012; Grear et al., 2016). However, lower densities of rats in rural environments may mean rats are less likely to have contact with other species, reducing the chances of infection. In the urban environment, rat populations occur at high concentrations, which may allow IAV to infect and spread within the population. Studies that have looked at rodent zoonotic pathogens in the urban environment have not reported IAV, and unbiased metagenomics studies of urban rats may have missed IAV due to the type of biospecimen analyzed, e.g., fecal pellets vs. oronasal swabs (Firth et al., 2014). We were unable to directly compare prevalence from fecal pellets and oronasal swabs, but detection of IAV from oronasal swabs in our study suggests this is an important site of the body to determine the presence of influenza in rats.

An influenza prevalence of 11.04% in the rats was unexpectedly high, given that wild rats had not been found to be PCR-positive for IAV before. This may or may not reflect the prevalence of IAV RT-PCR-positive rats across the entire city of Boston. Geographic clustering analyses revealed the Boston Public Garden and surrounding areas to be significantly underrepresented in terms of IAV RT-PCR-positive rats. While it is unknown why there were fewer IAV-positive rats in Boston Public Garden, the site is a public destination with widely dispersed waste receptacles and

TABLE 1 | Summary of real time-PCR-positive samples from rats.

Rat	Sex	Weight (g)	Specimen type	Original CT value	Passaged in ECEs	Post-passage PCR status	Post-passage CT value
42	Female	400	Oronasal	36.03	Yes	Negative	>45
44*	Female	300	Oronasal	35.24	Yes	Negative	>45
			Paw	36.23	Yes	Negative	>5
46	Male	400	Lung	35.93	Yes	Negative	>45
52	Male	225	Paw	37.85	No	–	–
53	Female	200	Oronasal	35.56	Yes	Negative	>45
59	Male	75	Oronasal	35.92	Yes	Negative	>45
65*	Male	150	Oronasal	36.94	Yes	Negative	>45
			Paw	35.89	Yes	Negative	>45
66	Male	90	Paw	36.90	yes	Negative	>45
71	Female	75	Paw	36.28	yes	Negative	>>45
72	Female	110	Oronasal	37.01	Yes	Negative	>45
75	Female	125	Paw	35.21	Yes	Positive	41.61
77	Male	125	Paw	42.69	No	–	–
87	Female	50	Paw	39.09	Yes	Negative	>45
94	Female	525	Oronasal	35.90	Yes	Negative	>45
98	Female	275	Paw	35.51	Yes	Negative	>45
104	Male	175	Oronasal	36.94	Yes	Negative	>45
218	Female	190	Oronasal	35.41	Yes	Negative	>45
220	Male	60	Lung	34.36	Yes	Negative	>45

*Both the original cycle threshold (CT) values as well as CT values following passage in embryonated chicken eggs are reported. *indicates the individual was positive for both oronasal and paw swabs.*

TABLE 2 | Best-fitting models explaining influenza A virus status of urban rats.

Model	Description			Likelihood ratio			AIC	
		k	*n*	log *L*	χ^2	*p*-value	Score	ΔAIC
1	Month+Geocluster*	3	160	−40.928	26.507	0.002	103.332	0.000
2	Month+Geocluster+Sex	4	154	−40.538	25.911	0.004	104.935	1.603
3	Month+Geocluster+Weight+Sex	5	154	−40.533	25.922	0.007	107.278	3.946
4	Month	2	160	−48.408	11.546	0.042	109.366	6.034
5	Geocluster	2	162	−53.152	6.727	0.151	116.688	13.356

Number of parameters (k) and number of observations (n) included in each model are reported. The statistical significance of each model is assessed with likelihood ratio tests summarized by the log likelihood (L), Chi squared (χ^2) and p-value. Models are ranked using Akaike's Information Criterion (AIC) summarized by the AIC score and AIC difference (ΔAIC). The top model "Month + Geocluster" (indicated by an asterisk) had the lowest AIC score and p-value.

frequent garbage collection. Moreover, this site is not home to a permanent human population, which may limit rodent access to human waste and refuse, an important resource for rats in residential neighborhoods.

Rats are able to compensate for decreased resource density by expanding their home range (Harper and Rutherford, 2016). This phenomenon is seen in rural rats as well as those living closer to farm buildings that have significantly smaller home ranges than those living on the margins of fields (Lambert et al., 2008). Thus, it seems plausible that fewer rat interactions occur as a function of decreasing resource density, and thus fewer opportunities for transmission or mechanical vectoring of IAV may occur in public spaces relative to the residential areas of Boston. This finding is consistent with studies of rural rats that found no evidence of IAV infection and suggests that IAV prevalence in rats may be a density-dependent phenomenon (Nettles et al., 1985; Grear et al., 2016).

Sampling month was also found to be significantly associated with IAV in rats, with prevalence being highest in the winter months. Experimental studies indicate that influenza persists outside the host for longer periods at lower relative humidity and low temperatures relative to high temperatures (Weber and Stilianakis, 2008). The environmental degradation of the virus particle may be an important limiting factor in the circulation of airborne transmission typical of mammalian hosts (Pica and Bouvier, 2012). Owing to an uneven sampling effort in our study resulting in a low sample size for some months, the power of this association is unclear and may only be resolved with an enhanced

study design that aims to sample rats consistently across all 12 months, rather than rely on convenience sampling.

The peak in influenza prevalence in winter observed in rats mimics the same seasonality of IAV in humans in temperate regions (Lofgren et al., 2007). However, without strain or subtype information, we cannot determine whether the seasonal pattern in rats is a reflection of endemic circulation, transmission from human sources (reverse zoonosis), or originates from other wild or domestic animals that occur in urban settings (birds, raccoons, pets, etc.). In support of the potential for reverse zoonosis, there is a growing body of literature that documents the transmission of human-origin pathogens in urban rats (Firth et al., 2014; Himsworth et al., 2015) and other peridomestic wildlife species such as skunks (Britton et al., 2010). In view of the increasing abundance of urban rodents, the incidence of zoonotic and reverse zoonotic transmission of IAV, as well as other pathogens, may become an important public health issue confronting cities.

Unfortunately, sequence data was not able to be obtained from IAV RT-PCR-positive rats in this study, likely due to very low viral titers in the original samples and degradation of viral genomes with repeated sample handling. The culturing of IAV from wild, non-avian hosts in embryonated chicken eggs (ECE) is a known challenge in the influenza field. Recent seasonal H3N2 viruses from humans have proven difficult to propagate in ECEs (Donis et al., 2014; Perez-Rubio and Eiros Bouza, 2018) and attempts to grow IAVs from marine mammals in ECEs are often unsuccessful (Puryear et al., 2016; Davis et al., in preparation). Propagation in chicken eggs depends upon the receptor binding affinity, fusion, and budding of the virus in ECEs, the concentration of virus, and the combination of both. While further passages in ECEs can result in viral isolation, using mammalian epithelial cell culture lines, such as MDCK cells, VERO cells, or in this case rat-derived epithelial cells may be beneficial in isolating and amplifying sufficient amounts of virus to be adequately sequenced (Donis et al., 2014; Perez-Rubio and Eiros Bouza, 2018). While it is unclear if rats are infected with IAV based exclusively on the molecular data presented here, the presence of viral nucleic acid in samples collected from the study population across field seasons and multiple swab sites is suggestive of replication within rats and transmission between conspecifics. Detection of viral RNA alone, however, is not conclusive evidence that the rats are truly infected. Rats may still be simply acting as mechanical vectors. This is supported by the fact that most IAV RT-PCR rats had either positive paw or oronasal swabs, but not both. Immunohistochemistry or immunofluorescence assays of infected tissue would support the role of rats acting as a host for the replication of influenza virus.

Isolation, culture, and sequencing of virus, or an unbiased metagenomics approach using oronasal or tracheal swabs, would be instrumental to learning more about the origin of these IAVs. It remains to be seen whether these viruses are rodent in origin and are endemic to rats, or if they are human in origin, picked up by rats living among urban waste. Lastly, the possibility exists that these viruses may be avian in origin given that the urban habitats where positive rats were detected are frequently shared with gulls, ducks, and pigeons. While viral sequences would be the best way to resolve this question, knowledge of rodent respiratory physiology may give us clues as to what IAV strains are most likely to affect rats. While the airway of rats has not been well characterized, the airway of mice has been (Ibricevic et al., 2006). In both human and mouse airways, α2,3-linked sialic acid receptors are found on ciliated cells and type 2 alveolar epithelial cells. These α2,3-linked sialic acid receptors preferentially bind avian IAV strains over human origin IAVs. However, unlike in humans where the α2,6-linked sialic acid receptor is expressed on both ciliated cells and goblet cells, mice have not been shown to express significant α2,6-linked sialic acid in their respiratory tract, which explains some of the difficulty in infecting mice with some human influenza strains. Assuming similar respiratory epithelial glycosylation in rats and mice, these findings suggest that the influenza strains infecting rats may not be the same as those affecting humans. Conversely, the fine detail of sialic acid linkages in the respiratory tract has proven increasingly complex, particularly with glycan array technology, and detailed mapping on rat epithelia is needed to make strong inferences about the ability of human influenza viruses to establish infection in rats in a wild setting.

The results of this study show that rats have been understudied as a potential reservoir for IAV, and that more work in this area is essential to understand the public health risks of rats and humans living at high density. To fully understand the role of rats in posing a health risk to humans or animals in an increasingly urbanized landscape, future studies should be directed at both isolating and sequencing the virus as well as larger-scale surveillance of rat populations in different urban centers.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of IACUC Policy on Use of Animal Cadavers and Animal Parts of Tufts University. The protocol was approved by the IACUC of the Tufts University Cummings School of Veterinary Medicine.

AUTHOR CONTRIBUTIONS

CC, JR, and MR designed the study. CC collected samples, necropsied rats, extracted RNA, performed PCRs, quantified data, and prepared this manuscript. NH extracted RNA, performed PCRs, egg inoculations, quantified data, and provided guidance. WP performed egg inoculations and provided guidance. JM provided guidance and space for necropsies. JL and JR provided guidance and funding to support this work. MR collected samples, necropsied rats, and provided both guidance

and funding to support this work. All authors contributed to the editing and finalizing of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00036/full#supplementary-material

Table S1 | The distribution of predictor/dependent variables tested with Generalized Linear Models: infection status, geocluster, sex and weight, throughout the course of the study (2016–2018).

Table S2 | The odds ratios associated with prevalence for each predictor variable tested with Generalized Linear Models: month, geocluster, sex and weight.

REFERENCES

Atkin, E., and Keizer, G. (2017). *America Is on the Verge of Ratpocalypse. The New Republic*. Available online at: https://newrepublic.com/article/144392/america-verge-ratpocalypse. (Accessed August 23, 2017).

Barry, J. M. (2004). The site of origin of the 1918 influenza pandemic and its public health implications. *J. Transl. Med.* 2:3. doi: 10.1186/1479-5876-2-3

Belser, J. A., Pulit-Penaloza, J. A., Sun, X., Brock, N., Pappas, C., Creager, H. M., et al. (2017). A novel A(H7N2) influenza virus isolated from a veterinarian caring for cats in a new york city animal shelter causes mild disease and transmits poorly in the ferret model. *J. Virol.* 91:e00672-17. doi: 10.1128/JVI.00672-17

Britton, A. P., Sojonky, K. R., Scouras, A. P., and Bidulka, J. J. (2010). Pandemic (H1N1) 2009 in Skunks, Canada. *Emerg. Inf. Infect. Dis.* 16, 1043–45. doi: 10.3201/eid1606.100352-1045

Donis, R. O., Chen, L. M., Davis, C. T., Foust, A., Hossain, M. J., Johnson, A., et al. (2014). Performance characteristics of qualified cell lines for isolation and propagation of influenza viruses for vaccine manufacturing. *Vaccine* 32, 6583–6590. doi: 10.1016/j.vaccine.2014.06.045

El-Sayed, A., Prince, A., Fawzy, A., Nadra-Elwgoud, Abdou, M. I., Omar, L., et al. (2013). Sero-prevalence of avian influenza in animals and human in Egypt. *Pak. J. Bio. Sci.* 16, 524–529. doi: 10.3923/pjbs.2013.524.529

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P., et al. (2014). Detection of zoonotic pathogens and characterization of novel viruses carried by commensal rattus norvegicus in New York city. *mBio* 5:e01933-14. doi: 10.1128/mBio.01933-14

Grear, D. A., Dusek, R. J., Walsh, D. P., and Hall, J. S. (2016). No evidence of infection or exposure to highly pathogenic avian influenzas in peridomestic wildlife on an affected poultry facility. *J. Wildlife Dis.* 53, 37–45. doi: 10.7589/2016-02-029

Harper, G., and Rutherford, M. (2016). Home range and population density of black rats (Rattus rattus) on a seabird island: a case for a marine subsidised effect? *N. Z. J. Ecol.* 40, 219–228. doi: 10.20417/nzjecol.40.25

Hill, N. J., and Runstadler, J. A. (2016). A bird's eye view of influenza A virus transmission: challenges with characterizing both sides of a co-evolutionary dynamic. *Integr. Comp. Biol.* 56, 304–316. doi: 10.1093/icb/icw055

Himsworth, C. G., Jardine, C. M., Parsons, K. L., Feng, A. Y. T., and Patrick, D. M. (2014). The Characteristics of Wild Rat (Rattus Spp.) populations from an inner-city neighborhood with a focus on factors critical to the understanding of rat-associated zoonoses. *PLoS ONE* 9:e91654. doi: 10.1371/journal.pone.0091654

Himsworth, C. G., Zabek, E., Desruisseau, A., Parmley, E. J., Reid-Smith, R., Jardine, C. M., et al. (2015). Prevalence and Characteristics of *Escherichia Coli* and *Salmonella Spp. in the feces of Wild Urban Norway and Black Rats (Rattus Norvegicus and Rattus Rattus) from an inner-city neighborhood of vancouver, Canada. J. Wildlife Dis.* 51, 589–600. doi: 10.7589/2014-09-242

Hinshaw, V. S., Bean, W. J., Webster, R. G., Rehg, J. E., Fiorelli, P., Early, G., et al. (1984). Are seals frequently infected with avian influenza viruses? *J. Virol.* 51, 863–865.

Ibricevic, A., Pekosz, A., Walter, M. J., Newby, C., Battaile, J. T., Brown, E. G., et al. (2006). Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. *J. Virol.* 80, 7469–80. doi: 10.1128/JVI.02677-05

Kothalawala, H., Toussaint, M. J. M., and Gruys, E. (2006). An overview of swine influenza. *Vet. Quart.* 28, 45–53. doi: 10.1080/01652176.2006.9695207

Lambert, M. S., Quy, R. J., Smith, R. H., and Cowan, D. P. (2008). The effect of habitat management on home-range size and survival of rural norway rat populations. *J. App. Ecol.* 45, 1753–61. doi: 10.1111/j.1365-2664.2008.01543.x

Lofgren, E., Fefferman, N. H., Naumov, Y. N., Gorski, J., and Naumova, E. N. (2007). Influenza seasonality: underlying causes and modeling theories. *J. Virol.* 81, 5429–36. doi: 10.1128/JVI.01680-06

McKenna, M. (2015). *Bird Flu Cost the US $3.3 Billion and Worse Could Be Coming*. Available online at: http://phenomena.nationalgeographic.com/2015/07/15/bird-flu-2/.(Accessed July 15, 2015).

Molinari, N. A. M., Ortega-Sanchez, I. R., Messonnier, M. L., Thompson, W. W., Wortley, P. M., Weintraub, E., et al. (2007). The annual impact of seasonal influenza in the US: measuring disease burden and costs. *Vaccine* 25, 5086–5096. doi: 10.1016/j.vaccine.2007.03.046

Nelson, M. I., Simonsen, L., Viboud, C., Miller, M. A., and Holmes, E. C. (2007). Phylogenetic Analysis reveals the global migration of seasonal influenza A viruses. *PLOS Path.* 3:e131. doi: 10.1371/journal.ppat.0030131

Nettles, V. F., Wood, J. M., and Webster, R. G. (1985). Wildlife surveillance associated with an outbreak of lethal H5N2 Avian influenza in domestic poultry. *Av. Dis.* 29, 733–741. doi: 10.2307/1590665

Parrish, C. R., Murcia, P. R., and Holmes, E. C. (2015). Influenza virus reservoirs and intermediate hosts: dogs, horses, and new possibilities for influenza virus exposure of humans. *J. Virol.* 89, 2990–2994. doi: 10.1128/JVI.03146-14

Perez-Rubio, A., and Eiros Bouza, J. M. (2018). Cell culture-derived flu vaccine: present and future. *Hum. Vac. Immunother.* 2018, 1–25. doi: 10.1080/21645515.2018.1460297

Pica, N., and Bouvier, N. M. (2012). Environmental factors affecting the transmission of respiratory viruses. *Curr. Opin. Virol.* 2, 90–95. doi: 10.1016/j.coviro.2011.12.003

Puryear, W. B., Keogh, M., Hill, N., Moxley, J., Josephson, E., Davis, K. R., et al. (2016). Prevalence of influenza A virus in live-captured North Atlantic gray seals: a possible wild reservoir. *Emer. Micr. and Inf.* 5:e81. doi: 10.1038/emi.2016.77

Runstadler, J., Hill, N. J., Hussein, I. T., Puryear, W., and Keogh, M. (2013). Connecting the study of wild influenza with the potential for pandemic disease. *Infect. Genet. Evol.* 17, 162–187. doi: 10.1016/j.meegid.2013.02.020

Shortridge, K. F., Gao, P., Guan, Y., Ito, T., Kawaoka, Y., Markwell, D., et al. (2000). Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective. *Vet. Micro*. 74,141–147. doi: 10.1016/S0378-1135(00)00174-7

Shriner, S. A., VanDalen, K. K., Mooers, N. L., Ellis, J. W., Sullivan, H. J., Root, J. J., et al. (2012). Low-pathogenic avian influenza viruses in wild house mice. *PLOS ONE* 7:e39206. doi: 10.1371/journal.pone.0039206

Spackman, E., Senne, D. A., Myers, T. J., Bulaga, L. L., Garber, L. P., Perdue, M. L., et al. (2002). Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *J. Clin. Microbio*. 40, 3256–3260. doi: 10.1128/JCM.40.9.3256-3260.2002

Taubenberger, J. K., Hultin, J. V., and Morens, D. M. (2007). Discovery and characterization of the 1918 pandemic influenza virus in historical context. *Antivir. Ther*. 12, 581–591.

U.S. Census Bureau; American Housing Survey (2015). *Boston - Housing Quality - All Occupied Units Table; generated by Charles Cummings; using American Housing Survey - Table Creator*. Available online at: https://www.census.gov/programs-surveys/ahs/data/interactive/ahstablecreator.html (Accessed January, 26 2017).

Weber, T. P., and Stilianakis, N. I. (2008). Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *J. Infect.* 57, 361–73. doi: 10.1016/j.jinf.2008.08.013

Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M., and Kawaoka, Y. (1992). Evolution and ecology of influenza A viruses. *Microbio. Mol. Bio. Rev*. 56, 152–179.

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Rat in a Cage: Trappability of Urban Norway Rats (*Rattus norvegicus*)

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Understanding the local ecology of urban Norway rats (*Rattus norevgicus*) is necessary to inform effective rat mitigation strategies. While Capture-Mark-Recapture (CMR) methods can be used to acquire such ecological information (e.g., abundance, movement patterns, and habitat use), these techniques assume that all individuals of the study population are equally trappable. To test whether urban rats adhere to this assumption, we conducted a 4-week CMR study in an urban neighborhood of Vancouver, Canada, to evaluate whether rat characteristics (i.e., age, sex, size, wound status, and infection with the pathogen *Leptospira* spp.) were associated with trappability. We found that the majority of rats entered traps in the first 2 weeks of trapping, and that larger rats were caught earlier in the trapping period. However, smaller, sexually immature rats were recaught more often than were larger, sexually mature rats, suggesting that prior capture affects the ability to recapture urban Norway rats. This highlights the need for CMR studies to account for size, sexual maturity, and prior capture when interpreting data.

Keywords: capture-mark-recapture method (CMR), ecology, Norway rat, *Rattus*, trappability, trapping bias, urban

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INTRODUCTION

Norway rats (*Rattus norvegicus*) are infamous urban exploiters, thriving in cities worldwide (Feng and Himsworth, 2014). Consequences of infestations can be severe. First, they are economically costly, estimated to account for over 19 billion dollars in damages annually in the United States through their consumption and contamination of food products alone (Pimentel et al., 2000). At the regional level, an estimate by the province of Alberta, Canada projected that rats would cost up to 42.5 million dollars annually in the absence of their current rodent control program (McClay et al., 2004). Second, rats place considerable pressures on ecosystems, contributing to global biodiversity loss both directly (e.g., through predation) and indirectly (e.g., through habitat modification and species displacement) (Towns et al., 2006; Jones et al., 2008; Doherty et al., 2016). Third, rats pose a health risk to human populations, harboring numerous zoonotic pathogens (those transmissible between animals and people) responsible for human morbidity and mortality in cities globally (Himsworth et al., 2013b).

An understanding of urban rat ecology is the cornerstone of any attempt to understand rats and rat-associated issues. To gain this knowledge, ecological methods such as Capture-Mark-Recapture (CMR) can be used to estimate population characteristics like abundance and density

(Wilson et al., 2007; Robinson et al., 2009; Sarmento et al., 2010), demographic characteristics (Votier et al., 2005; Lachish et al., 2007; Graham et al., 2013), and movement patterns (Beirinckx et al., 2006; Lagrange et al., 2014; Tuckey et al., 2017). However, traditional CMR techniques generally assume that all individuals are equally trappable over time (Krebs and Boonstra, 1984; Conroy and Carroll, 2009; Lindberg, 2012), an assumption which has not held in other species (e.g., Byrne et al., 2012; Carter et al., 2012; Camacho et al., 2017).

Although several models have been developed to address unequal detection of individuals within a population (reviewed by Gimenez et al., 2018), selecting and properly parameterizing an appropriate model is improved by information on trappability. Indeed, Abadi et al. (2013) stated that CMR models should incorporate animal characteristics that affect the probability of capture (e.g., sex, age) as covariates. When these covariates vary with time, termed "states" (e.g., reproductive status, or disease status), multi-state CMR models can be employed, which allow for individuals to transition among states (Gimenez et al., 2018). Further, where states relating to capture probability (i.e., trap aware or trap unaware) are affected by events (e.g., captured or not captured) multi-event models (an expansion of multi-state models; Pradel, 2005) can be used to more broadly reflect individual heterogeneity (Pradel and Sanz-Aguilar, 2012). Finally, when heterogeneity is not fully captured by covariates and states, individual heterogeneity can be modeled as individual random effects (Abadi et al., 2013). Therefore, it is important to understand the extent of capture heterogeneity within a population to first determine whether to incorporate it into CMR models (Ford et al., 2012), and second to identify an appropriate model and parameters.

The extent to which urban rats adhere to the assumption of equal trappability is largely unknown. Studies of their forest-dwelling conspecifics suggest that trap-related factors such as bait type (Laurance, 1992), trap type (Blackwell et al., 2002), and odors from previous occupants (e.g., predators and conspecifics) (Tobin et al., 1995; Parsons et al., 2015) may influence which individuals enter traps (i.e., trappability). Beyond trap-related effects—which can be controlled for through thoughtful study design (Williams et al., 2002)—trappability may vary with rat characteristics. For example, two capture-removal studies found that larger, sexually mature rats were more likely to enter traps early in a trapping period (Davis and Emlen, 1956; Himsworth et al., 2014a), countering assumptions of equal trappability. If the assumptions of CMR are not met, then this may lead to significant errors in the interpretation of the resulting data. For example, given that *Leptospira* spp., a zoonotic bacterial pathogen shed in rat urine, is more prevalent among larger, sexually mature rats (Himsworth et al., 2013a; Minter et al., 2017), the tendency for larger, sexually mature rats to enter traps earlier than other members of the population may result in inflated prevalence estimates for *Leptospira* spp.

Trappability may also be impacted by prior capture. Marked individuals can become "trap shy", whereby individuals avoid traps they have been caught in previously (Evans, 1951; Tanaka, 1963; King et al., 2003; Linhart et al., 2012), or "trap-happy", resulting in numerous recapture events (Geis, 1955; Morris, 1955; Tanaka, 1963; Gurnell, 1982). Although both Norway and black rats (*Rattus rattus*) may display neophobic behavior (Barnett, 1963; Clapperton, 2006), to our knowledge there has been only one other study to date which assessed how prior capture influences the trappability of Norway rats, and this study was performed in a rural setting (Tanaka, 1963).

The overarching objective of this study was to test the assumption of equal trappability of urban Norway rats. Specifically, we evaluated whether rat characteristics (i.e., age, sex, size, wound status, and infection with the pathogen *Leptospira* spp.) and prior capture were associated with trappability. This information will be valuable for future studies aimed at understanding rat ecology and rat-related issues (e.g., rat control, rat-associated public health risks, etc.).

METHODS

Trapping

Trapping was carried out in an urban neighborhood in Vancouver, British Columbia, Canada. Rats were trapped from June 2016–January 2017 in 31 proximal city blocks (**Figure 1**) which were selected as part of a larger CMR study (e.g., Byers et al., 2017; Donovan et al., 2018; Lee et al., 2018). Briefly, ten Tomahawk Rigid Traps (Tomahawk Live Traps, Hazelhurst, USA) were deployed in each city block. These traps were placed inside stainless-steel trap covers to prevent vandalism (Integrated Pest Supplies Ltd, New Westminster, Canada) and were chained to immovable objects along the length of the alleyway that bisected each city-block. To acclimatize rats to traps, traps were pre-baited for one week and fixed in an open position (Barnett, 1963). Bait consisted of peanut butter mixed with oats, and Hydrogel (ClearH2O, Westbrook, USA) was provided as a water source. Where possible, traps were placed against vertical surfaces in the path of potential rat runways (Himsworth et al., 2014a).

Following pre-baiting, active trapping commenced and continued for 4 weeks. Traps were set each evening by 16:00 and checked each morning by 07:00. Traps were set 5 days a week, and fixed open and baited on the sixth and seventh day. Traps were disinfected using 10% bleach immediately following any period of prebaiting (i.e., prior to active capture) as well as following any time a rat was captured to prevent the potential effect of odor on rat trappability [bleach and other disinfectants have been shown not to impact the trappability of various species of small mammals (Van Horn and Douglass, 2000; Wilson and Mabry, 2010)] and the unintended transmission of pathogens among rats (Health Canada, 2011).

Sampling

Trapped rats were transported to the back of a mobile laboratory-van, where each individually-caged animal was placed above a disinfected plastic tray and covered with a blanket to minimize stress. Urine was collected directly from the tray using a sterile syringe and was stored at −80°C until it was tested for *Leptospira* spp. Subsequently, rats were transferred into an inhalation induction chamber (Kent Scientific, Torrington, USA) and

Abbreviations: CMR, capture-mark-recapture.

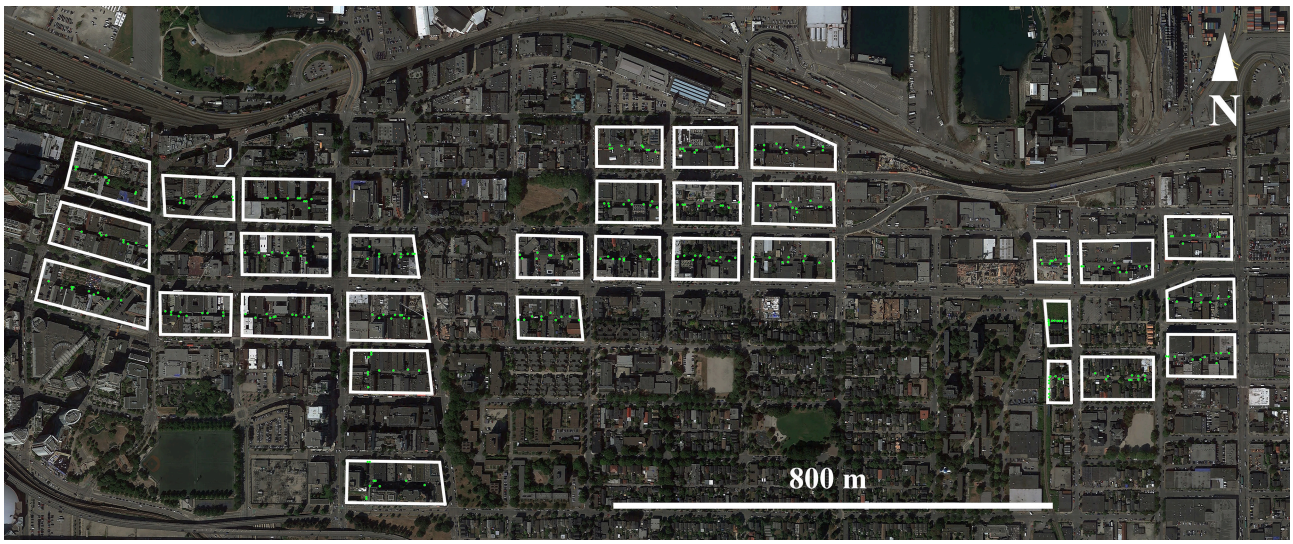

FIGURE 1 | Map of study sites in Vancouver, BC, Canada. Within each of the 31 city blocks where rats were trapped, green circles indicate the positions of the 10 traps placed in each block. Images provided through Google Earth Professional (https://www.google.com/earth/versions/#download-pro).

anesthetized with 5% isoflurane in oxygen using an isoflurane vaporizer (Associated Respiratory Veterinary Services, Lacombe, Canada). Anesthesia was maintained throughout sampling.

For each rat the following data were collected: body weight (grams), total length (nose-to-tip of tail in centimeters), sexual maturity (males with scrotal testes and females with a perforate vagina were considered mature), sex (male or female), and the presence/absence of bite wounds [presence determined as per (Himsworth et al., 2014b)]. Each rat was given a uniquely numbered laser-etched ear-tag (Kent Scientific, Torrington, USA) for identification upon recapture. Rats were allowed to recover fully from anesthesia (15–30 min) before being released at the exact location of their capture.

Occasionally there were too many rats caught for the field team to process. In those cases, rats that had not been processed by 1,600 h were counted but released without collection of additional data or sampling. The order in which rats were sampled was randomized by city-block each day.

Leptospira spp. Testing

Starting urine volumes ranged between 20 and 200 μL and all were volume corrected to 200 μL using sterile, 1X Phosphate Buffer Solution buffer, pH 7.4. Nucleic acid extraction and amplification of the LipL32 gene [encodes an outer membrane lipoprotein virulence factor (Stoddard et al., 2009) of pathogenic *Leptospira* species] was performed as outlined previously in Lee et al. (2018). Samples were classified as negative (cycle threshold [Ct]) $\geq$ 40, suspect positive (Ct = 37–39.99), or positive (Ct $\leq$ 36.99). Any sample within the suspect range was retested three times.

Statistics

Trap Success

To determine trap success, we divided the total number of rats caught by the total trap effort and adjusted according to Nelson and Clark (1973). This method accounts for the capture of non-target species and accidental trap activation by subtracting half a trapping unit from the total trap effort for each sprung trap.

Trappability

Linear regression was used to characterize the association between trap day (i.e., the day during the trapping period in which a rat was first captured, with "Day 1" being the first day of active trapping in any given city block) and the following covariates: sex, sexual maturity, weight, total length, bite wound presence, *Leptospira* spp. status, and season of capture (summer: June-August; and fall: September-November). Bivariable linear regression was used to individually examine the relationships between trap day and each characteristic and all covariates that were associated with trap day with a $p < 0.10$ were carried forward into a multivariable model. A backwards selection process was used to select the multivariable model with the lowest Akaike Information Criterion (AIC) to balance and compare relative model fit and parsimony. As weight and length were collinear (Spearman's Rho = 0.93, $p < 10^{-15}$), they were considered in separate competing models. Model assumptions were assessed in both the bivariable comparisons and in the final multivariable model. Biologically plausible interactions were assessed in the final model (i.e., between: weight and bite wounds; weight and sexual maturity; weight and sex).

Retrappability

Logistic regression was used to characterize the association between the aforementioned covariates and whether a rat was recaptured (yes/no) within seven days of their initial capture. A seven-day recapture window was chosen to ensure that every rat had an equal opportunity to be recaptured regardless of whether it was caught at the beginning or at the end of the trapping period. Note that 114 of 147 (78%) recaptured rats in the larger CMR study were recaught within seven days

of initial capture. Bivariable and multivariable modeling was carried out as described above; however, weight and length were dichotomized around their medians because they were not linearly associated with the log odds of the outcome. Biologically plausible interactions were assessed in the final model (i.e., between: weight and bite wounds; weight and sexual maturity; weight and sex).

Effect of the City-Block

To assess whether there was clustering of explanatory variables associated with the outcome by city-block, we compared the final multivariable model to the same model while including a random effect for the city-block, for both the *trappability model* (mixed effects linear regression) and the *retrappability model* (mixed effects logistic regression).

All analyses were carried out using R Studio version 1.1.456 (Boston, USA). Regression was performed using the stats (R Core Team, 2018) and lme4 (Bates et al., 2015) packages.

RESULTS

Altogether, 580 individual Norway rats were captured over 20 trap days with an overall trap success of 14%. Of the 580 Norway rats caught, 231 (39.8%) were caught in week one, 137 (23.6%) in week two, 106 (18.3%) in week three, and 106 (18.3%) in week four (**Figure 2**). Data were not collected for 195 rats (unmarked) because there were too many rats for the field team to process in one day. An additional six rats had missing data for one or more variables under consideration. A total of 379 rats were included in subsequent analyses.

Trappability

Among the 379 rats included for consideration, 195 (51%) were male (106 mature, 89 immature) and 184 (49%) were female (101 mature, 83 immature). The median weight and length of rats included in the trappability model were 111g and 30.5cm respectively. Urine was sampled from 335 individuals of which 39 (12%) were positive for *Leptospira* spp. (**Table 1**).

Upon bivariable linear regression (**Table 2**), weight ($p < 0.01$), length ($p < 0.01$), and wound presence ($p < 0.001$) were significantly ($p < 0.05$) associated with trap day. However, in the final multivariable model, only weight ($\text{beta}_{\text{adj}} = -0.0091$, 95% CI $= (-0.015, -0.0036)$, $p = 0.00134$) was retained, although length was roughly equivalent in that it explained approximately the same amount of variation in trap day ($R^2_{\text{length}} = 0.02626$; $R^2_{\text{weight}} = 0.02696$) (**Supplementary Figure 1**). In this model, heavier rats were more likely to be caught earlier in the trapping period. No interactions that were assessed were statistically significant in the final model.

Retrappability

In the first three weeks of trapping, 281 rats were released. Twenty-three rats were not released because they died either prior to or following anesthesia. While the cause of death for these individuals is unknown, some of these rats showed signs of rodenticide poisoning (i.e., bleeding from nose and mouth), malnutrition, and significant wounding (i.e., large open wounds). Indeed, rodenticide application was common in the study area and therefore it is highly probable that many of the captured rats had previously consumed rodenticides.

Seventy-three (26%) rats were recaptured, and 55 (75%) of these individuals were recaught within seven days of their initial capture. Of these, 46 were recaught once, seven were recaught twice (six immature and one mature rat), and two were recaught three times (both immature rats). Twenty-nine (53%) recaptured rats were male (13 mature, 16 immature) and 26 (45%) were female (9 mature, 17 immature) (**Table 1**). The median weight and length of rats included in the retrappability model were 80g and 27cm, respectively. Four of the recaptured rats (8%) tested positive for *Leptospira* spp. (**Table 1**).

Upon bivariable logistic regression (**Table 3**), the odds of being recaptured were significantly lower for rats that were mature ($p < 0.01$), that weighed 111 g or more ($p < 0.001$), and that were 30.5 cm or more in total length ($p < 0.01$). In the final multivariable model, only dichotomous weight was retained; however, while weight alone was the best predictor of whether a rat would be recaptured, the model containing length was roughly equivalent ($\text{AIC}_{\text{weight}} = 269.26$, $\text{AIC}_{\text{length}} = 271.34$). In this final model, larger rats were significantly less likely to be recaptured with rats heavier than the median weight having 0.34 times the odds of being recaptured as compared to rats less than the median weight (95% CI $= (0.18,0.62)$. No interactions that were assessed were statistically significant in the final model.

Effect of the City-Block

In the *Trappability Model*, adding the random effect of the block did not substantially change the effect of weight on trap day ($\text{beta}_{\text{adj}} = -0.009$, 95% CI $= (-0.15, -0.0031)$, $p < 0.01$). However, the variance associated with the effect of the block was 2.14 and the relative fit of the model increased slightly ($\text{AIC}_{\text{no random effect}} = 2350.9$, $\text{AIC}_{\text{random effect included}} = 2344.6$; **Supplementary Figure 2**). Similarly, in the *Retrappability Model*, adding the random effect of the block did not impact the effect of weight on recapture ($\text{OR}_{\text{adj}} = 0.34$, 95% CI $= (0.18,0.62)$, $p < 0.001$). Further, the variance associated with the effect of the block was 0 and the relative fit of the model decreased with the addition of the random effect ($\text{AIC}_{\text{no random effect}} = 269.26$, $\text{AIC}_{\text{random effect included}} = 271.3$).

DISCUSSION

Understanding the characteristics that influence urban rat trappability is essential to inform the design and interpretation of capture-based programs seeking to describe local rat ecology. We found that the number of rats captured decreased over the trapping period and that larger rats were more likely to enter traps earlier in the trapping period and were less likely to be recaught than were smaller rats. Together, these results suggest that urban Norway rats do not follow the assumption of equal trappability, and that CMR studies may be biased toward obtaining more robust capture histories for smaller individuals than larger individuals. Therefore, studies modeling CMR data for rats should consider distinct probabilities of capture based

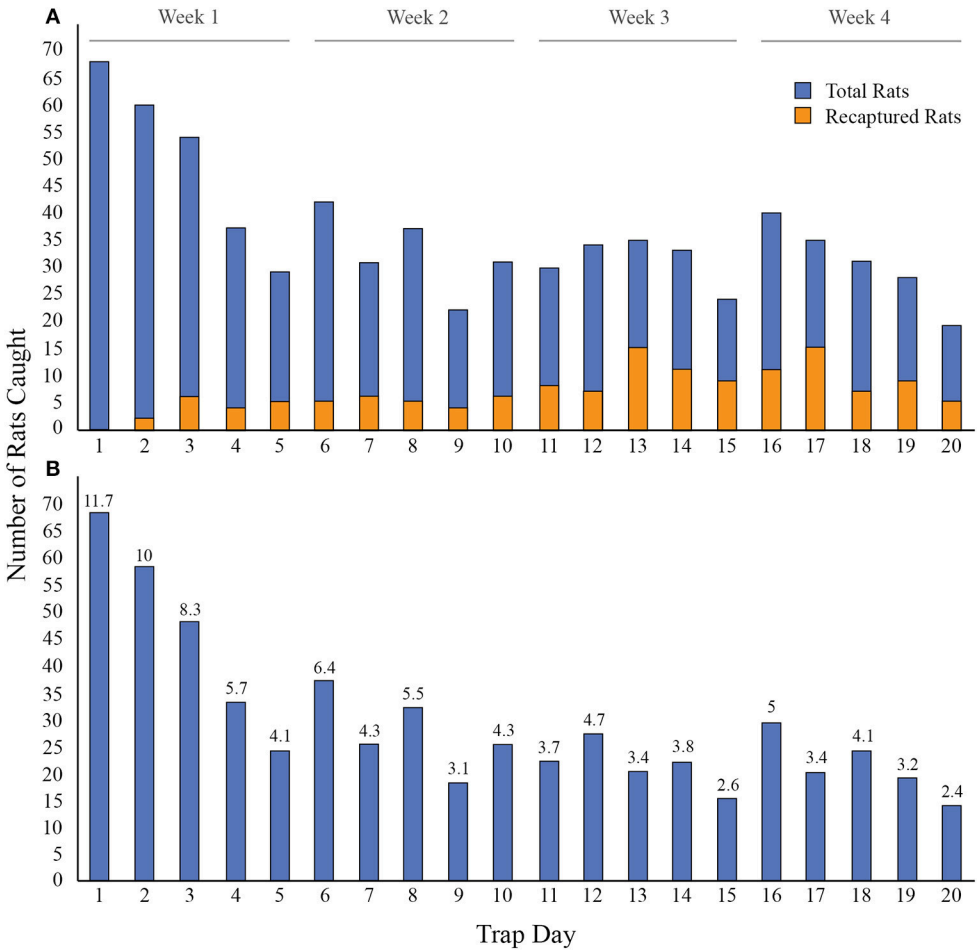

FIGURE 2 | Norway rats caught each day over 20 days in 31 city blocks of Vancouver, Canada. A. The number of rats caught each day, including rats caught only once (blue) and rats that were recaptured (orange), for a total of 720 rat capture events. B. The number of new rats caught each day (i.e. without recaptured rats). The percentage of rats caught out of the total number of individual rats (n = 580) is displayed on the top of each bar for each trap day (e.g., 11.7% of individuals were caught on day one).

on characteristics such as size and sexual maturity as well as differential impacts of prior capture on individual trappability.

Trapping Period

Trapping duration is an important component of CMR studies. Insufficient trapping periods may result in low sample sizes and thus affect accurate estimates of population characteristics (Olsen, 1975; Burke et al., 1995). However, extending trapping duration can be prohibitive due to equipment and labor costs. In this study, we found that trap success was greatest in the first week of trapping, with pronounced declines in rat numbers following the first day. These results are similar to a previous study which found that the greatest number of rats (22.2%) were caught on the first day of a 12-day trapping period (Himsworth et al., 2014a). Decreasing numbers of trapped rats using CMR could suggest that the proportion of "trap shy" individuals increases over the trapping period (Tanaka, 1963). Our results suggest that trapping initiatives may benefit from maximizing their efforts in the first week of trapping and highlights the importance of understanding how certain methods could increase trap success in that period (e.g., pre-baiting).

Trappability

Larger rats were more likely to enter traps early in the trapping period (**Supplementary Figure 1**). These results align with previous trap-removal studies which found that larger, sexually mature rats were more likely to enter traps in the first few days of trapping than were smaller, sexually immature rats (Davis and Emlen, 1956; Himsworth et al., 2014a). This is important because it suggests that short-term trapping-based studies seeking to describe local rat population ecology may be biased toward oversampling larger individuals. This is particularly problematic for studies evaluating the disease ecology and population health risks associated with urban rats because a number of zoonotic pathogens are associated with rat size (Glass et al., 1988; Himsworth et al., 2013a, 2014c). It is interesting therefore, that our study found no

TABLE 1 | Descriptive statistics of captured urban Norway rats (*Rattus norvegicus*).

Covariate	Levels	Not recaptured Total rats = 324 *N* (%total)	Recaptured Total rats = 55[a] *N* (%total)	Overall sample Total rats = 379 *N* (%total)
Sex	Female	158 (49)	26 (47)	184 (49)
	Male	166 (51)	29 (53)	195 (51)
Sexual Maturity	Immature	139 (43)	33 (60)	172 (45)
	Mature	185 (57)	22 (40)	207 (55)
Dichotomous Weight	<111g	153 (47)	36 (65)	189 (50)
	>=111g	171 (53)	19 (35)	190 (50)
Dichotomous Length	<30.5cm	151 (47)	35 (64)	186 (49)
	>=30.5cm	173 (53)	20 (36)	193 (51)
Wound Presence	None	250 (77)	43 (78)	293 (77)
	Present	74 (23)	12 (22)	86 (23)
Season	Summer (Jun-Aug)	131 (40)	22 (40)	153 (40)
	Fall (Sep-Nov)	193 (60)	33 (60)	226 (60)
Leptospira Status	Negative	248 (88) [b]	48 (92) [b]	296 (88) [b]
	Positive	35 (12) [b]	4 (8) [b]	39 (12) [b]

[a] *Only considered rats within the seven-week window for recapture (n = 281).*
[b] *Urine was not collected from all rats included in this analysis (n_{total} = 335, $n_{not\ recaptured}$ = 283, $n_{recaptured}$ = 52).*

TABLE 2 | Unadjusted (bivariable) and adjusted (multivariable) linear regression of each rat characteristic against the outcome of trap day; *N* = 379.

		Unadjusted[a]				Adjusted[b]			
Covariate	**Categories**	**Estimate[c]**	**SE[d]**	***p*-value**	**95% CI**	**Estimate**	**SE**	***p*-value**	**95% CI**
Sex	Female	Ref[e]	Ref	Ref	Ref	–	–	–	–
	Male	0.016	0.56	0.977	(−1.08,1.11)	–	–	–	–
Sexual Maturity	Immature	Ref	Ref	Ref	Ref	–	–	–	–
	Mature	−0.90	0.56	0.109	(−1.99,0.20)	–	–	–	–
Weight	Continuous	−0.0091	0.0028	0.0013	(−0.015, −0.0036)	−0.0091	0.0028	0.0013	(−0.015, −0.0036)
Length	Continuous	−0.12	0.038	0.0016	(−0.20, −0.047)	–	–	–	–
Wound Presence	None	Ref	Ref	Ref	Ref	–	–	–	–
	Present	−1.73	0.66	0.0091	(−3.03, −0.43)	–	–	–	–
Season	Summer (June–Aug)	Ref	Ref	Ref	Ref	–	–	–	–
	Fall (Sep–Nov)	0.50	0.57	0.38	(−0.62,1.62)	–	–	–	–
Leptospira Status	Negative	Ref	Ref	Ref	Ref	–	–	–	–
	Positive	−1.52	0.92	0.101	(−3.34,0.30)	–	–	–	–
R^2 for final model = 0.027									

[a] *Bivariable linear regression.*
[b] *Multivariable linear regression.*
[c] *Estimated effect of the given variable for a 1-day increase in trap day.*
[d] *Standard error.*
[e] *Reference category.*

association between trappability and carriage of *Leptospira* spp., even without controlling for characteristics such as weight. This suggests that carriage of *Leptospira* spp. may not be associated with the probability of capture. Finally, trappability was affected by some unmeasured block-level characteristic (e.g., resource availability). While our analysis was concerned with assessing the characteristics of individuals that entered traps earlier vs. later in a trapping period, it could also be that block-level characteristics influence the tendency of rats to enter traps.

Retrappability

While the number of new rats captured decreased with time, the number of recaptured individuals increased in the latter half of the trapping period. This is unsurprising, as the number of marked individuals available for recapture increases with the number of individuals marked. In this study, smaller rats were more likely to be recaught than larger rats. This is important because it suggests that prior capture may differentially affect trappability. Differences in aversion between smaller and larger rats may be due to differential access to resources (Feng and

TABLE 3 | Unadjusted (bivariable) and adjusted (multivariable) logistic regression of each rat characteristic against the outcome of binary recapture (yes/no).

Covariate	Categories	Unadjusted[a]				Adjusted[b]			
		OR[c]	SE[d]	*p*-value	95% CI	OR	SE	*p*-value	95% CI
Sex	Female	Ref[e]	Ref	Ref	Ref	–	–	–	–
	Male	1.20	0.30	0.55	(0.66,2.20)	–	–	–	–
Sexual Maturity	Immature	Ref	Ref	Ref	Ref	–	–	–	–
	Mature	0.37	0.31	0.0014	(0.20,0.68)	–	–	–	–
Weight	<111g	Ref	Ref	Ref	Ref	–	–	–	
	>=111g	0.34	0.32	0.00054	(0.18,0.62)	0.34	0.32	0.00054	(0.18,0.62)
Length	<30.5cm	Ref	Ref	Ref	Ref	–	–	–	–
	>=30.5cm	0.37	0.31	0.0015	(0.20,0.68)	–	–	–	–
Wound Presence	None	Ref	Ref	Ref	Ref	–	–	–	–
	Present	0.76	0.36	0.43	(0.36,1.49)	–	–	–	–
Season	Summer (June–Aug)	Ref	Ref	Ref	Ref	–	–	–	–
	Fall (Sep–Nov)	1.13	0.39	0.69	(0.62,2.08)	–	–	–	–
Leptospira Status	Negative	Ref	Ref	Ref	Ref	–	–	–	–
	Positive	0.47	0.55	0.18	(0.14,1.26)	–	–	–	–

The seven-day recapture window includes rats caught for the first time in the first three weeks of trapping; N = 281.
[a] *Bivariable logistic regression.*
[b] *Multivariable logistic regression.*
[c] *Odds ratio.*
[d] *Standard error.*
[e] *Reference category.*

Himsworth, 2014) whereby smaller, less dominant individuals are relegated to resources associated with increased risk (e.g., bait in traps). Indeed, experimental studies have demonstrated that adolescent rats (up to 60 days of age) display greater risk-taking behaviors than adults (Imhof et al., 1993). In addition to size, sexual maturity was associated with odds of recapture in the bivariable but not the multivariable model, suggesting that size likely represents more than just sexual maturity and that smaller, mature rats also have a decreased odds of recapture. This could be due to the relationship between an individual's body mass and their position within the social hierarchy of their colony, or as a general indicator of malnourishment, both of which could influence the results of CMR studies. Unlike with trappability, there did not appear to be an influence of block-level characteristics on retrappability. This may be due in part to dominance characteristics of rats which dictate rat interactions (Barnett, 1958). Dominance interactions may influence the tendency for subordinate rats to re-enter traps regardless of block-level characteristics. Finally, while there was no association between *Leptospira* spp. and retrappability, it is difficult to draw definitive conclusions given its overall low prevalence combined with the limited sample size for the recapture analysis.

Limitations

One potential limitation of the retrappability model is the restriction of designating rats as "recaught" only if they were recaught within seven days of their initial capture. This seven-day window was used in order to allow all rats an equal time to reenter traps. Although an initial assessment of the larger CMR dataset indicated that the majority of individuals were recaught within seven days, it could be that this timeframe biased our sample of recaught rats. For example, it is possible that larger rats require more time to overcome acquired trap shyness, and thus may be more likely to re-enter traps after seven days of capture. However, an analysis of the rats caught outside of the seven-day window demonstrated that these rats were on average 138g (n=16), within the weight range assigned to "smaller rats". Additionally, while our study found that 12% of rats were positive for *Leptospira* spp., it is possible that this is an underestimate of the actual number of infected rats as previous studies in in other species found intermittent or decreasing shedding of *Leptospira* over time (Leonard et al., 1992; Rocha et al., 2017). However, in a study which evaluated *Leptospira* shedding by Norway rats over two months in Salvador, Brazil, the bacteria was shed consistently over time (Costa et al., 2015), and therefore the extent to which variations in shedding affects our study is unclear. Finally, as there may be many rats in an area which never enter traps, it is important to note that our study can only make inferences on the "trappable" population of Norway rats, and may not be reflective of the entire population.

CONCLUSIONS

Overall, our study demonstrates that: (1) trap success is greatest at the start of a trapping period; (2) larger rats are more likely to enter traps early in the trapping period compared to smaller rats; and (3) smaller rats are more likely to reenter traps than larger rats. This is important because it indicates that urban Norway rats violate the assumption of equal trappability inherent to traditional CMR methods. We suggest that studies employing these methods consider rat characteristics as well as the impact of capture during study design, CMR model selection, and data interpretation.

DATA AVAILABILITY

Datasets are available upon request. The data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

All procedures and protocols for this study were approved by the University of British Columbia's Animal Care Committee (A14-0265) and are in accordance with the Canadian Council on Animal Care's national guidelines.

AUTHOR CONTRIBUTIONS

This study was conceived of by KB, ML, and CH. Sample collection and data analysis was performed by KB, ML, and JB. Writing of the initial draft of the manuscript was performed by KB and ML with substantial contributions from CH. All authors contributed to revising the manuscript. Funding was acquired by KB and DP.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00068/full#supplementary-material

REFERENCES

Abadi, F., Botha, A., and Altwegg, R. (2013). Revisiting the effect of capture heterogeneity on survival estimates in capture-mark-recapture studies: does it matter? *PLoS ONE* 8:e62636. doi: 10.1371/journal.pone.0062636

Barnett, S. A. (1958). An analysis of social behaviour in wild rats. *Proc. Zool. Soc. Lond.* 130, 107–152. doi: 10.1111/j.1096-3642.1958.tb00565.x

Barnett, S. A. (1963). *The rat: A study in behaviour*. Chicago, IL: Aldine Publishing Co.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Beirinckx, K., Van Gossum, H., Lajeunesse, M. J., and Forbes, M. R. (2006). Sex biases in dispersal and philopatry: insights from a meta-analysis based on capture–mark–recapture studies of damselflies. *Oikos* 113, 539–547. doi: 10.1111/j.2006.0030-1299.14391.x

Blackwell, G. L., Potter, M. A., and McLennan, J. A. (2002). Rodent density indices from tracking tunnels, snap-traps and fenn traps: do they tell the same story? *N. Z. J. Ecol.* 26, 43–51.

Burke, V. J., Greene, J. L., and Whitfield Gibbons, J. (1995). The effect of sample size and study duration on metapopulation estimates for slider turtles (*Trachemys scripta*). *Herpetologica* 51, 451–456. doi: 10.2307/3892770

Byers, K. A., Lee, M. J., Donovan, C. M., Patrick, D. M., and Himsworth, C. G. (2017). A novel method for affixing Global Positioning System (GPS) tags to urban Norway rats (*Rattus norvegicus*): feasibility, health impacts and potential for tracking movement. *J. Urban Ecol.* 3:e68496. doi: 10.1093/jue/jux010

Byrne, A. W., O'Keeffe, J., Green, S., Sleeman, D. P., Corner, L. A. L., Gormley, E., et al. (2012). Population estimation and trappability of the European badger (*Meles meles*): implications for tuberculosis management. *PLoS ONE* 7:e50807. doi: 10.1371/journal.pone.0050807

Camacho, C., Canal, D., and Potti, J. (2017). Lifelong effects of trapping experience lead to age-biased sampling: lessons from a wild bird population. *Anim. Behav.* 130, 133–139. doi: 10.1016/j.anbehav.2017.06.018

Carter, A. J., Heinsohn, R., Goldizen, A. W., and Biro, P. A. (2012). Boldness, trappability and sampling bias in wild lizards. *Anim. Behav.* 83, 1051–1058. doi: 10.1016/j.anbehav.2012.01.033

Clapperton, B. K. (2006). A review of the current knowledge of rodent behaviour in relation to control devices. *Sci. Conserv.* 263, 1–55.

Conroy, M. J., and Carroll, J. P. (2009). "Capture–mark–recapture studies for estimating abundance and density," in *Quantitative Conservation of Vertebrates* (Oxford: Wiley-Blackwell), 135–159.

Costa, F. E., Wunder, E. A. Jr., De Oliveira, D., Bisht, V., Rodrigues, G., Reis, M. G., et al. (2015). Patterns in *Leptospira* shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission. *PLoS Negl. Trop. Dis.* 9:e0003819. doi: 10.1371/journal.pntd.0003819

Davis, D. E., and Emlen, J. T. (1956). Differential trapability of rats according to size and sex. *J. Wildl. Manag.* 20, 326–327. doi: 10.2307/3796975

Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G., and Dickman, C. R. (2016). Invasive predators and global biodiversity loss. *Proc. Natl. Acad. Sci. U.S.A.* 113, 11261–11265. doi: 10.1073/pnas.1602480113

Donovan, C. M., Lee, M. J., Byers, K. A., Bidulka, J., Patrick, D. M., and Himsworth, C. G. (2018). Leptospira spp. in the oral cavity of urban brown rats (*Rattus norvegicus*) from vancouver, Canada—implications for rat-rat and rat-human transmission. *J. Wildl. Dis.* 54, 635–637. doi: 10.7589/2017-08-194

Evans, F. C. (1951). Notes on a population of the striped ground squirrel (*Citellus tridecemlineatus*) in an abandoned field in southeastern Michigan. *J. Mammal.* 32, 437–449. doi: 10.2307/1375792

Feng, A. Y. T., and Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosyst.* 17, 149–162. doi: 10.1007/s11252-013-0305-4

Ford, J. H., Bravington, M. B., and Robbins, J. (2012). Incorporating individual variability into mark-recapture models. *Meth. Ecol. Evol.* 3, 1047–1054. doi: 10.1111/j.2041-210X.2012.00243.x

Geis, A. D. (1955). Trap response of the cottontail rabbit and its effect on censusing. *J. Wildl. Manag.* 19, 466–472. doi: 10.2307/3797457

Gimenez, O., Cam, E., and Gaillard, J.-M. (2018). Individual heterogeneity and capture-recapture models: what, why and how? *Oikos* 127, 664–686. doi: 10.1111/oik.04532

Glass, G. E., Childs, J. E., Korch, G. W., and Leduc, J. W. (1988). Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus*

norvegicus). *Epidemiol. Infect.* 101, 459–472. doi: 10.1017/S0950268800054418

Graham, J., Smith, G. C., Delahay, R. J., Bailey, T., McDonald, R. A., and Hodgson, D. (2013). Multi-state modelling reveals sex-dependent transmission, progression and severity of tuberculosis in wild badgers. *Epidemiol. Infect.* 141, 1429–1436. doi: 10.1017/S0950268812003019

Gurnell, J. (1982). Trap response in woodland rodents. *Acta Theriol.* 27, 123–137. doi: 10.4098/AT.arch.82-10

Health Canada (2011). *Pathogen Safety Data Sheets: Infectious Substances – Leptospira Interrogans*. Available online at: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/leptospira-interrogans-material-safety-data-sheets-msds.html

Himsworth, C. G., Bidulka, J., Parsons, K. L., Feng, A. Y. T., Tang, P., Jardine, C. M., et al. (2013a). Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of vancouver, Canada. *PLoS Negl. Trop. Dis.* 7:e2270. doi: 10.1371/journal.pntd.0002270

Himsworth, C. G., Jardine, C. M., Parsons, K. L., Feng, A. Y. T., and Patrick, D. M. (2014a). The characteristics of wild rat (*Rattus* spp.) populations from an inner-city neighborhood with a focus on factors critical to the understanding of rat-associated zoonoses. *PLoS ONE* 9:e91654. doi: 10.1371/journal.pone.0091654

Himsworth, C. G., Parsons, K. L., Jardine, C., and Patrick, D. M. (2013b). Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne Zoonotic Dis.* 13, 349–359. doi: 10.1089/vbz.2012.1195

Himsworth, C. G., Patrick, D. M., Mak, S., Jardine, C. M., Tang, P., and Weese, J. S. (2014c). Carriage of *Clostridium difficile* by wild urban Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*). *Appl. Environ. Microbiol.* 80, 1299–1305. doi: 10.1128/AEM.03609-13

Himsworth, C. G., Zabek, E., Tang, P., Parsons, K. L., Koehn, M., Jardine, C. M., et al. (2014b). Bacteria isolated from conspecific bite wounds in Norway and black rats: implications for rat bite–associated infections in people. *Vector Borne Zoonotic Dis.* 14, 94–100. doi: 10.1089/vbz.2013.1417

Imhof, J. T., Coelho, Z. M. I., Schmitt, M. L., Morato, G. S., and Carobrez, A. P. (1993). Influence of gender and age on performance of rats in the elevated plus maze apparatus. *Behav. Brain Res.* 56, 177–180. doi: 10.1016/0166-4328(93)90036-P

Jones, H. P., Tershy, B. R., Zavelta, E. S., Croll, D. A., Keitt, B. S., Finkelstein, M. E., et al. (2008). Severity of the effects of invasive rats on seabirds: a global review. *Conserv. Biol.* 22, 16–26. doi: 10.1111/j.1523-1739.2007.00859.x

King, C. M., Davis, S. A., Purdey, D., and Lawrence, B. (2003). Capture probability and heterogeneity of trap response in stoats (*Mustela erminea*). *Wildl. Res.* 30, 611–619. doi: 10.1071/WR02091

Krebs, C. J., and Boonstra, R. (1984). Trappability estimates for mark-recapture data. *Can. J. Zool.* 62, 2440–2444. doi: 10.1139/z84-360

Lachish, S., Jones, M., and McCallum, H. (2007). The impact of disease on the survival and population growth rate of the Tasmanian devil. *J. Anim. Ecol.* 76, 926–936. doi: 10.1111/j.1365-2656.2007.01272.x

Lagrange, P., Pradel, R., Bélisle, M., and Gimenez, O. (2014). Estimating dispersal among numerous sites using capture-recapture data. *Ecology* 95, 2316–2323. doi: 10.1890/13-1564.1

Laurance, W. F. (1992). Abundance estimates of small mammals in Australian tropical rainforest: a comparison of four trapping methods. *Wildl. Res.* 19, 651–655. doi: 10.1071/WR9920651

Lee, M. J., Byers, K. A., Donovan, C. M., Bidulka, J. J., Stephen, C., Patrick, D. M., et al. (2018). Effects of culling on *Leptospira interrogans* carriage by rats. *Emerging Infect. Dis.* 24, 356–360. doi: 10.3201/eid2402.171371

Leonard, F. C., Quinn, P. J., Ellis, W. A., and O'Farrell, K. (1992). Duration of urinary excretion of leptospires by cattle naturally or experimentally infected with *Leptospira interrogans* serovar hadjo. *Vet. Rec.* 131, 435–439. doi: 10.1136/vr.131.19.435

Lindberg, M. S. (2012). A review of designs for capture–mark–recapture studies in discrete time. *J. Ornithol.* 152, S355–S370. doi: 10.1007/s10336-010-0533-9

Linhart, P., Fuchs, R., Polákov,á, S., and Slabbekoorn, H. (2012). Once bitten twice shy: long-term behavioural changes caused by trapping experience in willow warblers *Phylloscopus trochilus*. *J. Avian. Biol.* 43, 186–192. doi: 10.1111/j.1600-048X.2012.05580.x

McClay, A. S., Fry, K. M., Korpela, E. J., Lange, R. M., Roy, L. D., and Alberta Research Council (2004). "Costs and threats of invasive species to Alberta's natural resources," in *Alberta Sustainable Resource Development*, (Edmonton, AB), 1–122.

Minter, A., Diggle, P. J., Costa, F., Childs, J., Ko, A. I., and Begon, M. (2017). Evidence of multiple intraspecific transmission routes for *Leptospira* acquisition in Norway rats (*Rattus norvegicus*). *Epidemiol. Infect.* 70, 1–11. doi: 10.1017/S0950268817002539

Morris, R. F. (1955). Population studies on some small forest mammals in eastern Canada. *J. Mammal.* 36, 21–35. doi: 10.2307/1375718

Nelson, L., and Clark, F. W. (1973). Correction for sprung traps in catch/effort calculations of trapping results. *J. Mammal.* 54, 295–298. doi: 10.2307/1378903

Olsen, R. W. (1975). Length of trapping period in population studies. *J. Mammal.* 56, 696–697. doi: 10.2307/1379487

Parsons, M. H., Sarno, R. J., and Deutsch, M. A. (2015). Jump-starting urban rat research: conspecific pheromones recruit wild rats into a behavioral and pathogen-monitoring assay. *Front. Ecol. Evol.* 3:146. doi: 10.3389/fevo.2015.00146

Pimentel, D., Lach, L., Zuniga, R., and Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *Bioscience* 50, 53–65. doi: 10.1641/0006-3568(2000)050[0053:EAECON]2.3.CO;2

Pradel, R. (2005). Multievent: an extension of multistate capture-recapture models to uncertain states. *Biometrics* 61, 442–447. doi: 10.1111/j.1541-0420.2005.00318.x

Pradel, R., and Sanz-Aguilar, A. (2012). Modelling trap-awareness and related phenomena in capture-recapture studies. *PLoS ONE* 7:e32666. doi: 10.1371/journal.pone.0032666

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/.

Robinson, S. J., Waits, L. P., and Martin, I. D. (2009). Estimating abundance of American black bears using DNA-based capture-mark-recapture models. *Ursus* 20, 1–11. doi: 10.2192/08GR022R.1

Rocha, B. R., Narduche, L., Oliveira, C. S., Martins, G., and Lilenbaum, W. (2017). Molecular demonstration of intermittent shedding of *Leptospira* in cattle and sheep and its implications on control. *Cienc. Rural* 46:e2017088. doi: 10.1590/0103-8478cr20170088.

Sarmento, P. B., Cruz, J. P., Eira, C. I., and Fonseca, C. (2010). Habitat selection and abundance of common genets *Genetta genetta* using camera capture-mark-recapture data. *Eur. J. Wildl. Res.* 56, 59–66. doi: 10.1007/s10344-009-0294-z

Stoddard, R. A., Gee, J. E., Wilkins, P. P., McCaustland, K., and Hoffmaster, A. R. (2009). Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagn. Microbiol. Infect. Dis.* 64, 247–255. doi: 10.1016/j.diagmicrobio.2009.03.014

Tanaka, R. (1963). On the problem of trap-response types of small mammal populations. *Res. Popul. Ecol.* 5, 139–146. doi: 10.1007/BF02518838

Tobin, M. E., Engeman, R. M., and Sugihara, R. T. (1995). Effects of mongoose odors on rat capture success. *J. Chem. Ecol.* 21, 635–639. doi: 10.1007/BF02033706

Towns, D. R., Atkinson, I. A. E., and Daugherty, C. H. (2006). Have the harmful effects of introduced rats on islands been exaggerated? *Biol. Invasions* 8, 863–891. doi: 10.1007/s10530-005-0421-z

Tuckey, T. D., Fabrizio, M. C., Norris, A. J., and Groves, M. (2017). Low apparent survival and heterogeneous movement patterns of invasive blue catfish in a coastal river. *Mar. Coast. Fish.* 9, 564–572. doi: 10.1080/19425120.2017.1381207

Van Horn, R. C., and Douglass, R. J. (2000). Disinfectant effects on capture rates of deer mice (*Peromyscus maniculatus*). *Am. Midl. Nat.* 143, 257–260. doi: 10.1674/0003-0031(2000)143[0257:DEOCRO]2.0.CO;2

Votier, S. C., Hatchwell, B. J., Beckerman, A., McCleery, R. H., Hunter, F. M., Pellatt, J., et al. (2005). Oil pollution and climate have wide-scale impacts on seabird demographics. *Ecol. Lett.* 8, 1157–1164. doi: 10.1111/j.1461-0248.2005.00818.x

Williams, B. K., Nichols, J. D., and Conroy, M. J. (2002). *Analysis and Management of Animal Populations: Modeling, Estimation, and Decision Making*. San Diego, CA: Academic Press.

Wilson, D. J., Efford, M. G., Brown, S. J., Williamson, J. F., and McElrea, G. J. (2007). Estimating density of ship rats in New Zealand forests by capture-mark-recapture trapping. *N. Z. J. Zool.* 31, 47–59. doi: 10.2307/24058126

Wilson, J. A., and Mabry, K. E. (2010). Trapping mammals in a cautious world: the effect of disinfectants on trap success. *West. N. Am. Nat.* 70, 467–473. doi: 10.3398/064.070.0406

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A Case Study of Two Rodent-Borne Viruses: Not Always the Same Old Suspects

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Two Old World rodents, house mice (*Mus musculus*) and Norway rats (*Rattus norvegicus*), were introduced into and established populations on every continent, save Antarctica. With their travels, they concomitantly introduced several zoonotic agents capable of causing human diseases. Two viruses—Lymphocytic choriomeningitis virus (LCMV; genus *Arenavirus* with mice) and Seoul virus (SEOV; genus *Hantavirus* with rats)—can cause chronic infections within their respective rodent hosts, resulting in persistent or life-long sporadic shedding of virus through secreta and excreta. Although the prevalence of infection within their wild rodent hosts can exceed 25% among mice infected with LCMV and 50% among rats infected with SEOV, acute human disease resulting from direct transmission from wild rodents is rarely reported even though both species live in close coexistence with humans. The usual "classic" zoonotic cycle of transmission from wild rodent reservoirs to humans now includes multiple unusual/unexpected routes. The largest described outbreaks of human disease caused by these viruses are linked to pet rodents. A novel reservoir host, the golden hamster, has supplanted house mice as the major source of LCMV infection, and SEOV outbreaks are linked to fancy rats kept as pets. Following LCMV, and to a lesser extent SEOV, outbreaks or infections associated with lab animals and/or cultured tissues derived from mice and hamsters have led to hundreds of cases of LCMV among laboratory workers, and SEOV has been detected among cell-cultured tissues. Additionally, LCMV is now a recognized source of severe congenital disease and is the unexpected source of severe and often fatal disease among solid organ recipients. Although the extensive usual and unusual routes of LCMV infection are exceptional there are many parallels with SEOV emergence.

Keywords: lymphocytic choriomeningitis virus, Seoul virus, *Rattus norvegicus*, *Mus musculus*, zoonotic disease

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INTRODUCTION

The critical role of zoonoses in the emergence of new infectious diseases impacting humans and/or domestic animals has reached a level of near doctrine. Associated with this perspective has been an in-depth effort to study the dynamics of pathogens in reservoir populations with less focus on the significant role of the dynamics of the target (human or domestic animal) population in changing patterns of transmission. Previously, one of the authors emphasized the roles of adaptation and

changes in social/behavioral activities that could qualitatively change risk patterns (Childs, 2004). However, relatively little focus continues to be paid to these factors.

As an example of what we believe is a critically understudied aspect of emerging diseases, we review the evolution of risk patterns from two viral rodent-borne zoonoses during the past 80 years. These agents, lymphocytic choriomeningitis virus (LCMV) and Seoul virus (SEOV), are among the earliest and best studied agents. Historically, the house mouse (*Mus musculus*) and the Norway rat (*Rattus norvegicus*) have been recognized as their reservoirs. Close contact with wild populations of these rodents has been viewed as the major risk factor for humans. During the past several decades, however, as research has progressed, a more nuanced understanding of how human activities have altered human risk has developed.

In this paper we outline the history of the discoveries of these agents, the diseases they cause, why they persist in their reservoir populations and how human activities and unforeseen events widened our understanding of the epidemiology of these viruses and the spectrum of people at risk for infection.

HISTORY OF LYMPHOCYTIC CHORIOMENINGITIS (LCM) AND LCM VIRUS (LCMV)

Lymphocytic choriomeningitis (LCM) was first described as a cause of aseptic meningitis among hospitalized patients in St. Louis, Missouri, USA, in the early 1930s (Armstrong and Lillie, 1934; Rivers and Scott, 1935). Although rarely reported as of 2018, LCMV was once a significant cause of aseptic meningitis. Hospital-based studies conducted from 1953 to 1958 identified LCM among 8% of cases of encephalitis among 713 hospitalized patients (Meyer et al., 1960). Infection, however does not always cause disease among humans, and clinical cases appear rare among the general population.

Lymphocytic choriomeningitis virus (LCMV) is a bi-segmented, negative sense RNA virus (Bishop and Auperin, 1987). The two gene segments are labeled L (7.5 kb) and S (3.5 kb). LCMV is the type species of the family *Arenaviridae* which includes Lassa, Junin, Machupo, and Sabia—the latter four viruses are closely related arenaviruses causing hemorrhagic disease among humans. Over the years, significant genetic heterogeneity and phenotype of clinical course have been shown to vary with different LCMV isolates. However, numerous LCMV variants can cause severe disease among humans (Amman et al., 2007; Emonet et al., 2007; Palacios et al., 2008) and significant genetic variation occurs among isolates (Albariño et al., 2010). LCMV has served as a model system for understanding the immunobiology of virus infections (Zhou et al., 2012), and in 1996 Nobel prizes were awarded to two individuals (Peter Doherty and Rolf Zinkernagel) who used LCMV as a model to enumerate the immunobiology of virus persistence and differential immune responses based on modes of transmission.

In humans acute disease is marked by fever, headache, myalgia and other non-specific signs and symptoms typical of many viral infections (Farmer and Janeway, 1942; Biggar et al., 1975a,b; Folk et al., 2011). LCM presents without the lymphopenia and thrombocytopenia characteristic of severe disease caused by closely related arenaviruses associated with hemorrhagic fever. In LCM cerebral fluid pleocytosis, choriomeningitis and other neurological signs and symptoms develop among some patients after an apparent remission of acute symptoms.

Acute LCM is not a mandated reportable disease and few cases are identified as of 2018. Without any doubt this infection and disease is significantly underestimated, in part because of limited facilities offering diagnostic testing (Barton et al., 1995; Barton and Hyndman, 2000).

DIAGNOSIS OF LCM

The most commonly used tests for identifying LCMV infections among humans and animals are serological assays that detect antibodies to the virus in serum and cerebral spinal fluid. The ELISA (enzyme-linked immunosorbent antibody test) is cheap to perform, and may be followed by a confirmatory immuno-fluorescent antibody test (IFA) or plaque reduction neutralization assay (PRNT), which is the gold standard of serological tests that distinguishes among different arenaviruses (Armstrong and Lillie, 1934; Rivers and Scott, 1935; Traub, 1936a; Wooley et al., 1939; Fischer et al., 2006; Becker et al., 2007; Takimoto et al., 2008; Knust et al., 2011). Currently, RT-PCR detection is available at specialty labs.

Antibody assays can detect both IgM (recent or ongoing infection) and IgG (infection at some time); a four-fold increase in IgG antibody titer between two sampling dates is diagnostic of recent infection. However, serum and intrathecal levels (measured in cerebral spinal fluid—CSF) of LCMV antibody are always low but in suspected cases, even a low titer of LCMV antibody aids the diagnosis (Sukthana, 2006). The neutralization test requires live virus and is available only in specialty labs because of the obvious risk of human exposure and infection among unsuitably protected lab personnel.

The incidence of infection among humans is unknown (Centers for Disease Control and Prevention, 2006). On-site diagnostic testing for LCMV infection is limited among laboratories serving acute care hospitals, as exemplified by a survey in which none of 30 such facilities contacted in Connecticut, United States, had the in-house means of testing. In the same survey most infectious disease doctors (28 responses out of 35 sent) would consider LCM in a differential diagnosis if there was a history of contact with wild mice or a healthy or sick pet rodent; only six would consider LCMV as a potential cause of unexplained fever in immunocompromised patients without an exposure history (Centers for Disease Control and Prevention, 2006).

EPIDEMIOLOGY OF LCM

The routes of LMCV transmission to humans are believed to include fine particle aerosols arising from infected secreta and excreta (saliva and urine most commonly), droplets,

fomites (e.g., contaminated bedding of laboratory, commercial colonies or pet rodents), bites and contact with rodent blood. Exact routes of transmission of LCMV from mice to humans have not been evaluated—in part because large house mouse-associated outbreaks do not occur—but aerosol transmission is most probable.

LCMV infection acquired from pet rodents, notably the Syrian golden hamster (see sections below), provide significant evidence of aerosol, droplet, or fomite transmission of LCMV. In some of these occurrences multiple family members have become infected, some of whom reported no physical contact with the animal (Biggar et al., 1975a,b, 1977). Many laboratory infections of humans appear to be airborne (Armstrong et al., 1969; Vanzee et al., 1975; Hotchin et al., 1977). Sentinel guinea pigs housed in the same room but separated from infected mice show a high susceptibility to airborne infection and experimental studies have demonstrated high susceptibility of mice and guinea pigs to airborne exposure with LCMV (Benda, 1964).

LCMV presents little risk of infection to general populations. Various serologic tests indicate a prevalence of antibody of 1–5% of persons (Childs et al., 1991; Stephensen, 1992; Childs and Wilson, 1994; Knust et al., 2011), although it is unclear how many of these persons suffered from acute disease. It is likely that the prevalence of antibody would be greater among certain populations if surveyed. In some locations severe mouse infestations, and many LCMV-infected mice, are present in low-socioeconomic status (SES) urban locations containing substandard housing with inadequate sanitation and waste removal. The same features provide an excellent habitat for brown rats (*R. norvegicus* see sections on Seoul virus below: **Figure 1A**) (Childs et al., 1991, 1992). Early studies linking house mice to LCM often described heavy mouse infestations.

Immunocompromised individuals or children born with an *in utero* infection acquired from an acutely infected mother are at the highest risk of developing severe and fatal disease (see below).

ENTER THE HOUSE MOUSE (*MUS MUSCULUS*)

Shortly after the discovery of the disease, evidence rapidly accumulated implicating the house mouse as the reservoir host of LCMV transmitted to humans (Armstrong and Sweet, 1939) (see **Figures 2A,B** for an abbreviated timeline of some significant observations marking the history of LCMV). A year prior to the discovery of LCMV in wild house mice, laboratory colonies of albino mice were found infected. Additional research on these colonies elucidated the different phenology of vertically acquired (*in utero*) and horizontal intra-specific infection with regard to immunity, duration of virus persistence and shedding, and the pathological consequences of infection (the significance of vertical transmission is discussed below) (Traub, 1936a, 1938, 1939).

The widespread introduction and global colonization by the house mice (Long, 2003) suggest that LCMV could enjoy a cosmopolitan distribution, and some authors suggest this (Charrel and de Lamballerie, 2010). However, most studies indicate that the distribution of infected mice is patchy and tends to cluster as discussed below. There are four subspecies of the genus *Mus* but herein we refer to them collectively as house mice or *M. musculus*.

Most available LCMV isolates come from the United States and West-Central Europe (Albariño et al., 2010), but reports of infection and isolation of LCMV are confirmed from Argentina (Sabattini et al., 1970, 1974), Japan (Morita et al., 1991), and variants of LCMV based on RNA sequencing have been reported from Australia (Dandenong virus) and Africa (Kudoko virus) (Lecompte et al., 2007; Palacios et al., 2008). As of 2009, no clinical cases of LCM had been described from Southeast Asia (Kim et al., 2009).

Describing/discovering a zoonotic reservoir for agents pathogenic to humans and transmitted by inter-species contact is old. The association between rabies, dog bite, and human disease dates to at least the year 500 B.C. (Steele et al., 1991). However, the observation that laboratory mice, in addition to wild mice, were infected with LCMV was significant as disease outbreaks occurred among research personnel working or in contact with lab mice (**Figure 2A**). Additionally, these findings necessitated reconsideration of published scientific reports based on mouse models (potentially contaminated by LCMV) and raised serious concerns over the health and sanitation of lab mouse colonies and the risk of infection for research staff and suppliers. Even short periods, such as 30 min, of contact between infected and susceptible mice kept within the same cage was sufficient for transmission to occur (Skinner and Knight, 1973). The "natural" history of LCMV maintenance and transmission to humans included only the normal suspects, albino, and wild house mice at this juncture in what developed into a far more complicated and unusual history (**Figure 2A**).

OF MICE AND MONKEYS

Humans are not the only primates in which mice derived from breeding stock of commercial vendors were the source of infection and severe disease. In 1981, two outbreaks of infectious hepatitis of unknown origin were reported from zoos in the United Kingdom and United States affecting marmosets and tamarins (Family: Callithricidae)—New World primates of which several of the affected spp. are considered endangered (Montali et al., 1989). Between 1981 and 1989, 12 outbreaks were reported from different zoos in the USA and this new, unique and fatal disease was named Callithrichid hepatitis (Montali et al., 1989).

Follow-up studies showed that inoculations of liver homogenates from an ill monkey into three common marmosets produced severe disease within 10 days; all monkeys either required euthanasia or died from infection. Known viruses capable of causing hepatitis in non-human primates were ruled out and the etiologic agent was unidentified (Montali et al., 1989). In 1991, the causative agent was identified as LCMV (Stephensen et al., 1991) and one outbreak was linked to a point source involving perinatal mice ("pinkies") fed to these monkeys as a supplemental source of animal protein (Montali et al., 1993). In the same study, two veterinarians who autopsied infected

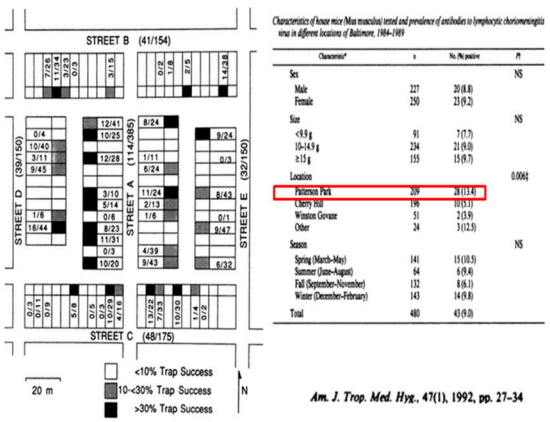

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TABLE 1

Demographic characteristics and prevalence of infections with LCMV and SEOV in Baltimore residents

	Antibody			
	LCMV	n	SEOV	n
Prevalence	4.70	1,149	0.25	1,180
Race				
African-American	4.73	1,079	0.27	1,110
White	3.28	61	0	61
Other	11.11	9	0	9
Sex				
Male	4.76	820	0.35	845
Female	4.56	329	0	335
Age				
≤20	3.25	369	0	379
21–30	4.62	563	0.52	580
31–40	7.30	178	0	183
>40	7.90	38	0	38

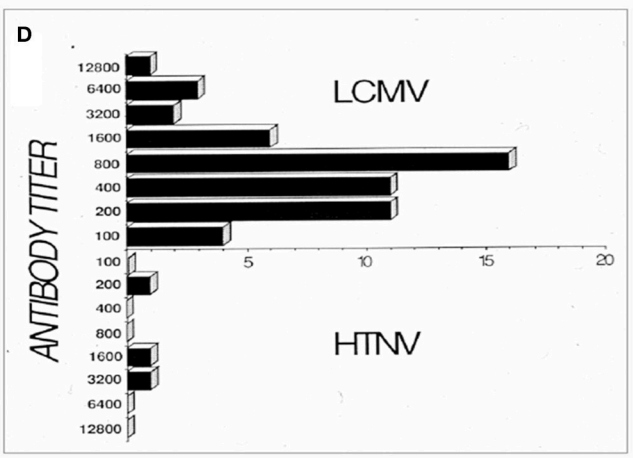

FIGURE 1 | (A) Multiple scenes from alleys in Baltimore, MD, from which rats were captured to elucidate the natural history of SEOV (dark arrows indicate sites of extensive rat burrows). House mice were captured from row houses fronting on the alleys to study the epidemiology of LCMV. Mice were never captured in alleys and few rats were captured within residences. **(B)** Map on left shows the layout of one square block of row houses surrounding an alley shown in **(A)**. The shading indicates mouse-trap success, an index of population density, obtained over a three-year period. The table to the left shows the prevalence of antibodies to LCMV among house mice captured among several study sites, indicating clustering of infection within blocks. **(C)** The prevalence of antibodies among individuals visiting an STD clinic in Baltimore, of which many resided in low-SES neighborhoods likely to harbor extensive Norway rat populations. The prevalence of LCMV antibodies increases with age, but the lack of sufficient data on SEOV precluded any similar conclusions. **(D)** Endpoint titers of antibody-positive sera to LCMV or SEOV.

monkeys and one individual bitten by an infected monkey had seroconverted to LCMV.

EPIDEMIOLOGY OF LCMV AMONG HOUSE MICE

The prevalence of LCMV infection among wild mouse populations has been reported to range from 0 to 25% (**Figures 1C,D**) (Stephensen, 1992; Childs and Peters, 1993; Childs and Wilson, 1994; Becker et al., 2007; Knust et al., 2011; Williams et al., 2018). LCMV infection of house mice can be highly focal at both large and small scales (see Childs and Peters, 1993). In Baltimore, Maryland, United States, house mouse densities and LCMV infection varied extensively among connected row houses studied in a two-block area (**Figure 1B**). Prevalence of LCMV infection varied between 50 and ∼100% among mice in some houses while neighboring residences had few or no infected mice—observations also obtained from other studies (Childs et al., 1992; Emonet et al., 2007). There was a significant association between mouse density (trap success) and prevalence of LCMV at different sampling sites, which could reflect density-dependent horizontal transmission or clustering of chronically infected mice and vertical transmission (**Figure 1B**) (Childs et al., 1992).

At regional scales the distribution of LCMV is patchy. From 1960 to 1962, in possibly the most intensive effort to map LCMV distribution among wild house mice, 1795 house mice were collected from 376 evenly spaced trapping sites in the Federal Republic of Germany. Although the overall prevalence of infection was 3%, all 65 LCMV-positive mice came from a subset of 44 trapping sites (Ackermann et al., 1964).

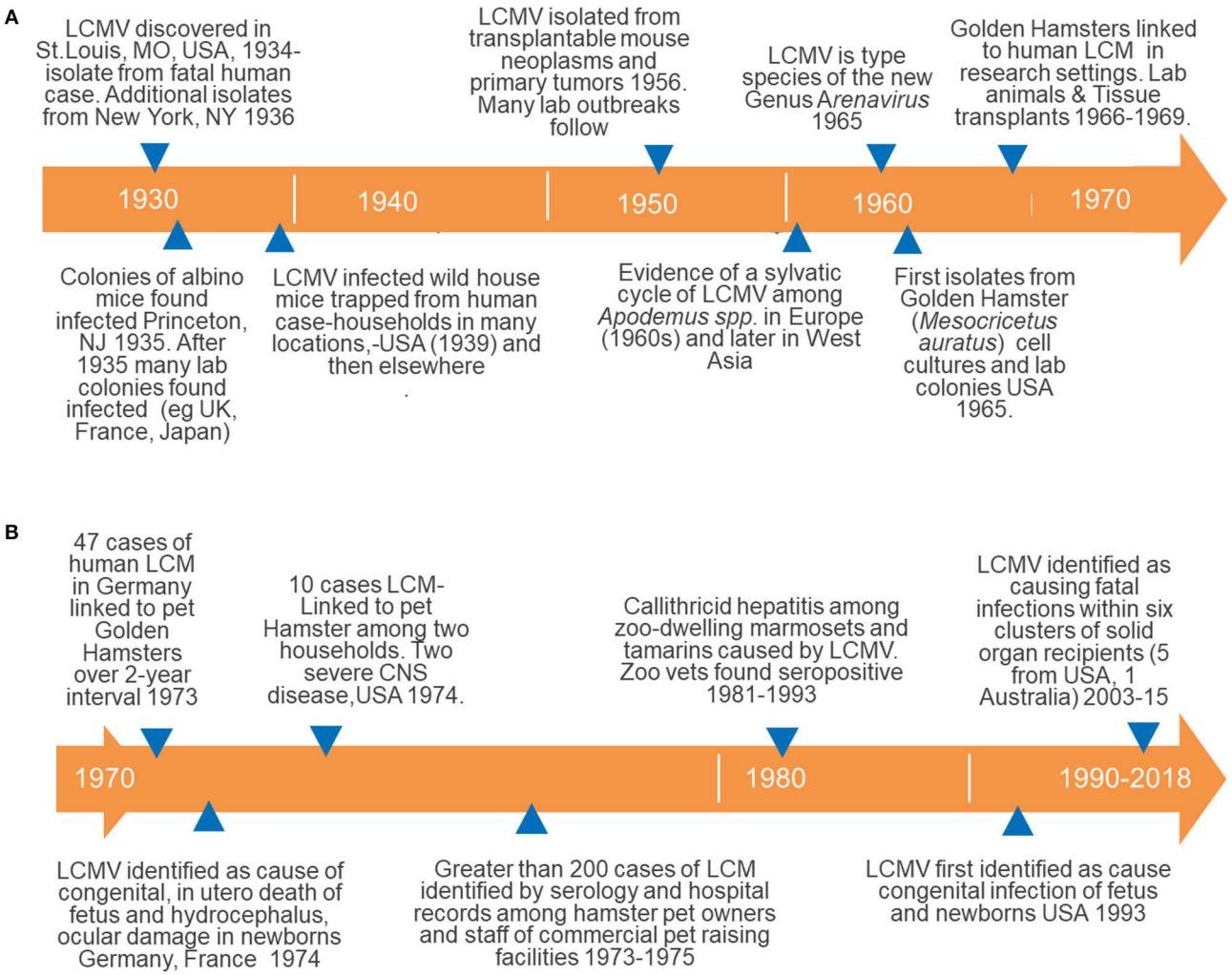

FIGURE 2 | Timelines documenting the history of major events in our understanding of the epidemiology maintenance and transmission of LCMV to humans; **(A)** from the discovery of LCMV (1930s) and its association with wild house mice through the discovery of human-to-human transmission causing severe congenital disease; **(B)** details of the emergence of a transcendent host for LCMV, the golden hamster, through the recognition of LCMV among recipients of solid organ transplants. These figures illustrate the development of both usual and unusual sources of LCMV infection among humans as discussed in detail in the text.

Due to this patchiness of LCMV infections among house mice, negative findings are important and further illuminate the spotty distribution of this virus. In New York City, New York, United States, highly sensitive genetic methods failed to identify any LCMV among 395 mice captured from several sites (Williams et al., 2018). However, human LCM occurs within the city (Asnis et al., 2010).

The focality of LCMV infections among mice in some settings may be due to the combination of congenital transmission LCMV among mice (Lehmann-Grube, 1963, 1971) and the demic structure of house mouse populations (e.g., Petras, 1967; Singleton, 1983). Urban mice have an extremely limited dispersal distance when occupying human structures, such as row houses (Baltimore) and apartments (New York) (it is greater among mice inhabiting agricultural settings) (Pocock et al., 2005). Genetic studies of house mice populations found that mice inhabiting adjacent houses were closely related but mice sampled from houses on different blocks were genetically distinguishable (Murphy et al., 2005).

Persistent chronic infection of mice results in *in utero* vertical transmission of LCMV to fetuses and surviving offspring so that infected mice never clear the virus. Infection prevalence among such progeny can approach 100% (Traub, 1936c; Lehmann-Grube, 1963, 1971). Most importantly for virus transmission to humans, persistently infected mice shed LCMV in their saliva and urine throughout their lives (Traub, 1936a,b; Barber et al., 2006), generating material that can lead to aerosol infection within infested households. Infected laboratory mice appear to suffer no ill effects from their infection. However, tolerance of persistent LCMV infection is not absolute as deposits of antibody-virus complexes in the kidneys of older lab mice can lead to severe glomerulonephritis as antigen-antibody complexes accumulate in kidneys (see Buchmeier et al., 1980). This occurrence has not been shown among wild house mice,

which probably do not live long enough to suffer from this late-onset complication.

House mice born from an uninfected dam can be infected later in life by horizontal transmission routes. These individuals develop antibodies, clear their infections and are believed not to become chronic shedders of LCMV, although demonstration of this phenomenon has not been confirmed by longitudinal study of wild mice.

Although viruses have evolved multiple mechanisms to escape the host immune system, of relevance to this review are the mechanisms viruses use to suppress antiviral defenses. The most well-established model system is LCMV infection of house mice. The Armstrong strain of LCMV is readily cleared from immunocompetent adult mice; conversely, LCMV Clone 13 differs from Armstrong (i.e., the parental strain) by 2 amino acid positions and causes persistent infection in adult mice (Salvato et al., 1988, 1991; Ahmed et al., 1991). Persistence of LCMV Clone 13 is associated with functional impairment (often referred to as "exhaustion") and deletion of virus-specific $CD8^+$ T cells, increased production of IL-10, and induction of programmed death (PD)-1 activity (Moskophidis et al., 1993; Barber et al., 2006; Ejrnaes et al., 2006). Blocking the IL-10R or PD-1/PD-L1 pathways promotes LCMV clearance (Barber et al., 2006; Brooks et al., 2006; Ejrnaes et al., 2006; Maris et al., 2007). The exhaustion phenotype of CD8+ T cells during persistent LCMV infection is mediated by Treg cells because depletion of Treg cells increases the expansion of LCMV-specific CD8+ T cells, but has no effect on virus persistence (Penaloza-MacMaster et al., 2014).

OTHER WILD RODENT HOSTS

Other sylvatic rodents have been shown to be infected with LCMV. Species of *Apodemus* infected with LCMV in Europe were identified as early as the 1950s and continue to be found to the present (**Figure 2A**; for descriptions of early findings see (Lehmann-Grube, 1971, 1984). Exposure to *Apodemus* mice and cases of LCM among humans have not been conclusively identified.

BACK TO THE RESEARCH LABORATORY

The next chapter in the unusual history of LVMC began in the 1940s−1950s, when scientific investigators identified LCMV among transplantable mouse tumors and laboratory cell lines—including a line used for growing rabies virus for vaccines (**Figure 2A**: see Hotchin, 1971; Hotchin et al., 1977 for review). Clearly this unanticipated route of human exposure was not one of the usual routes of transmission thought previously to be restricted to contact with living rodents (**Figure 1A**). Additionally, in 1992 LCMV laboratory personnel working with nude mice were shown to be LCMV-positive by serology (Dykewicz et al., 1992).

Within a year, other observations indicated that infected and infectious cell cultures derived from an additional species, the Syrian or golden hamster (*Mesocricetus auratus*), were responsible for human laboratory infections (**Figure 2A**) (Lewis et al., 1965; Hotchin, 1971). But not only cell cultures were incriminated, as over the course of 2 years, 1971–1973, 48 staff members of the University of Rochester Medical Center were sickened by LCM acquired by working directly with hamsters (**Figure 2A**, unusual because the first infections arising from hamsters came from cultured bits rather than the whole animal (Vanzee et al., 1975; Hotchin et al., 1977).

These findings were alarming enough but the public health problem was quickly shown to extend beyond sporadic reports of infection/disease in laboratorians. Reports of pet hamster-associated LCM were identified in the early 1970s when 47 cases from pet hamsters were reported over 2 years in Germany (**Figure 2B**) (Ackermann et al., 1972). Within 3 years, 1973–1975, serological and clinical studies identified pet hamster-associated LCM among 181 persons living in 12 states in the United States. Many of these cases were identified through a national alert issued by the CDC after investigating an initial cluster of cases among pet owners (summarized within Gregg, 1975). Other reports continued to surface over years from a number of countries.

These findings heralded in the highly unusual emergence of a secondary host for LCMV that was associated closely with humans as a laboratory animal and household pet (Skinner et al., 1976). Most significantly, the size of outbreaks associated with hamsters dwarfs outbreaks and sporadic occurrences of LCM associated with mice by many fold. A novel reservoir host had supplanted the natural house mouse reservoir as the major link to human infection and disease, transforming itself from its status of "not the usual suspect" to one of "the usual suspects."

A BRIEF NATURAL HISTORY OF HAMSTERS

Hamsters are native to much of Europe and Asia (Wilson and Reeder, 2005). Of the over 20 spp. of hamsters, only five are kept as pets with the most popular being the golden hamster. As with the house mouse they are introduced species to the New World, although their introduction was intentional.

The first lab colony of golden hamsters was derived from wild-caught animals in ∼1930 and housed at Hebrew University, Jerusalem. From this initial colony these readily domesticated animals made their way into many research settings, including the United States, after the first established commercial breeding facilities appeared in the 1940s (Adler, 1948). Almost simultaneously, hamsters were marketed as adorable household pets. Based on a survey conducted by the American Veterinary Association in 2012, the number of households in the United States owning a pet hamster was estimated at 877,000, with 1,146,000 individuals owned (American Veterinary Medical Association, 2012).

ENTER HUMAN-TO-HUMAN TRANSMISSION

Of major significance to human health are two other twists in LCMV's mechanisms of transmission to humans associated

with severe and fatal disease. Unlike many of the arenaviruses causing hemorrhagic fevers, LCMV had not been shown to be transmitted from human to human. Acquired LCMV infection from contact with a reservoir, including cultured cells, rarely causes human severe disease or fatalities; however, the discovery of *in utero* infection of the fetus from Europe beginning in 1974 changed this perception (**Figure 2B**) (Ackermann et al., 1972). This was a clear and unusual departure from previously described transmission routes (**Figure 2A**). When acute infection of pregnancy occurs during the first trimester of pregnancy, symptomatic *in utero* and/or perinatal infection results in significant neurological disease, such as hydrocephalus, microcephaly, hydrops fetalis, and choriomeningitis. Surviving young suffer from permanent sequelae. This significant development in the changing epidemiology of LCM was not described until almost 20 years later in 1990s in the United States (van den Pol, 2006; Bonthius and Perlman, 2007; Kang and McGavern, 2008; Meritet et al., 2009).

Pregnant women rarely show any indication of acute LCM. Presumably, their immunosuppressed state predisposes them to acute infection which can then be transmitted to the fetus. Support for this observation comes from reports of infection and severe and often fatal disease among other immunosuppressed persons, such as organ recipients (see below) (**Table 1**). That this virus is an important, but underestimated, cause of fetal infection is likely accurate as diagnostic testing is restricted. However, only 58+ incidents of congenital LCM have been reported up to 2010, and the TORCH agents are ascribed to most cases.

Many authors now refer to LCM as an "emerging disease" or "re-emerging disease" (Armstrong and Sweet, 1939; Barton et al., 2002; Fischer et al., 2006; Jamieson et al., 2006; Asnis et al., 2010; Barton, 2010). In the case of LCMV, most of the emerging was due to recognition of congenital disease rather than an increase in infection/disease among the general public.

ANOTHER NOVEL AND DISTURBING DEVELOPMENT

In 2003 and again in 2005, the medical community was taken by surprise by the identification of severe and most often fatal LCM among transplant patients (**Table 1**; **Figure 1B**) [see Perspective (Peters, 2006) for additional comments about the evolution of LCMV transmission to humans]. Not only was this heretofore undiscovered transmission route of epidemiologic importance, it was a clear demonstration that LCM goes undiagnosed in acute disease or infection and is certainly significantly underestimated. Donors had to be acutely infected as, unlike in mice, the virus does not persist. Thus, the organ donor became a new transmission source and a very unusual suspect.

In total, five clusters of LCM derived from donors in the United States have resulted in recipients with 21 cases and 15 deaths (**Table 1**). One survivor suffered severe neurological sequelae (Mathur et al., 2017). An additional cluster of donor-derived LCM was discovered in Australia in 2008 when three solid organ recipients died of LCM (**Table 1**). The LCMV typed by genetic analyses in Australia was a variant previously undocumented. Australia had been reported clear of LCMV up until this discovery (Palacios et al., 2008). How most organ donors acquired their LCMV infection is undetermined. However, in 2005, LCM in four recipients (two died) was traced to transmission from an organ donor who kept golden hamsters as pets. These animals were purchased from a major supplier of hamsters. Traceback studies identified infected hamsters within the store from which the animal originated as well as the distribution and commercial breeding location.

HOW RARE IS ORGAN TRANSPLANT-ASSOCIATED LCM?

In 2017 there were 19,484 kidney, 8,082 liver, and 2,449 lung transplants performed in the USA—the solid organs responsible for LCMV infections resulting from infected donors (**Table 1**) (https://www.organdonor.gov/statistics-stories/statistics.html).

Risk of LCMV among organ recipients is low. As individual hospitals and organizations procure and provide donor organs, there are an unstandardized variety of tests performed on tissues. Recommendations for live donor pathogen screening by the Organ Procurement and Transplantation Network do not include testing for LCMV (Rosen and Ison, 2017).

These alarming occurrences further confirm that the once usual and originally described source of LCMV transmission to humans through contact with the introduced house mouse has been supplanted by contact with another non-native rodent species, the hamster.

CONTROL OF LCMV AND PREVENTION OF LCM

Against the usual suspect, the house mouse, the most effective methods to reduce the risk of human infection with LCMV are to employ traditional and, less rarely implemented but most effective, integrated pest management, to control and prevent transmission from the usual suspects. These combine rodent elimination through trapping and poisoning, sound construction procedures or remediation as needed, and reduced access to human-generated food resources through sanitary efforts (Childs and Wilson, 1994; Centers for Disease Control and Prevention, 2005), and are essentially the same measures for reducing SEOV exposure and disease (see sections below).

Controlling exposure to LCMV from laboratory animals and derived cell cultures requires barrier breeding, periodic testing of rodent colonies and cultures of derived tissues, prevention of wild mouse ingression and culling of any colony shown to be infected, as the role of wild mice in infecting laboratory colonies has been well-established for decades (**Figure 2B**) (see Skinner et al., 1977 for review) (https://www.criver.com/sites/default/files/resources/LymphocyticChoriomeningitisVirusTechnicalSheet.pdf).

In addition to testing samples derived from colonies of mice, sampling of rodent bedding has been suggested as a means of surveillance. However, in one significant case, testing of material failed to identify LCMV contamination of lab mice, which was

TABLE 1 | Six clusters of solid organ-associated LCMV infection; five from the United States and one from Australia.

Date/location/reference	Donor condition	Organ transplanted	Recipient outcome (time to death days)
2003/USA/(Fischer et al., 2006)	Apparent head trauma	Kidney	Died (53)
		Kidney	Died (76)
		Liver	Died (17)
		Lung	Died (9)
2005/USA/	No reported disease	Kidney	Died (23)
		Liver	Died (26)
		Lung	Died (23)
		Kidney	Survived*
2008/Australia/(Palacios et al., 2008)	Cerebral Hemorrhage	Kidney	Died (36)
		Liver	Died (30)
		Kidney	Died (29)
2008/USA/(Centers for Disease Control and Prevention, 2008)	Cardiac Arrest -2° to encephalitis	Kidney	Died (∼28)
		Kidney	Died (∼70)
2011/USA/(MacNeil et al., 2012)	Cerebral edema	Kidney	Died (30)
		Kidney	Survived**
		Liver	Survived***
		Lung	Died (20)
2013/USA/(Centers for Disease Control and Prevention, 2014)	Cerebral Hemorrhage	Liver	Survived/Critically ill****
		Kidney	Died (20)****
		Kidney	Survived****
		Cornea	Survived/Asymptomatic****

Details of the time of occurrence, evidence of donor illness, tissue transplanted, and outcome of recipients of organs are summarized.
**Ribavirin administered day 26 after LCMV discovered as cause.*
***Treated with IV acyclovir (2X) and oral valacyclovir.*
****No antivirals administered.*
*****Immunosuppression was promptly reduced and ribavirin and/or intravenous immunoglobulin therapy were initiated.*

revealed only after embryo transplants, which also demonstrated a failure of caesarian delivery to control spread (Ike et al., 2007).

Control of LCMV among hamsters requires radically different approaches when animals are destined for the pet trade. Studies have shown control to be problematic even when recommendations (sealing buildings from wild mice entry, regular veterinary inspections, monitoring for zoonotic infections, culling of animals, etc.) to reduce infection among breeding and distribution centers exist—although they are not always strictly adhered to or followed (Amman et al., 2007). As of 2018, the risk of human infection with LCMV among persons working in breeder facilities is still substantial. In one study, 32% ($N = 97$) of workers had antibodies to LCMV and LCM was diagnosed among four persons (Knust et al., 2014). Of over 1,800 mice tested, the prevalence of LCMV infection was >20% and RT-PCR identified currently infected mice. Complete culling of colonies was implemented.

Although a rare phenomenon, the problems associated with preventing LCMV transmission through solid organ transplant are formidable. The organizations providing organs have different operational standards for screening donors and none screen for LCMV infection (MacNeil et al., 2012). Additionally, screening is unlikely as it is not considered cost effective. New occurrences of transplant-associated LCM will continue to occur, although rarely (this also applies to other viruses shown to cause solid organ transplant-associated encephalitis, including West Nile virus and rabies virus).

Some suggestions for screening donors for history of contact with wild or pet rodents have been made, but given the backlog of waiting recipients and pressure to obtain organs, the effectiveness of such efforts will be difficult to assess (MacNeil et al., 2012). Screening of possibly rejected donors for LCMV infection would be a necessary component to such a measure, and in past investigations (Fischer et al., 2006; Fischer, 2008) most donors showed no evidence of acute LCMV infection and were only demonstrated to be infected after the fact.

LCM CONCLUSIONS

The original concept of the zoonotic cycle leading to human LCM was through contact with wild mice and inhalation of their aerosolized excreta/secreta. From the 1930s onward, it was well-recognized that LCMV was maintained among mice by vertical and horizontal transmission among mouse populations (**Figure 2A**). The first twist in this traditional cycle was when LCM outbreaks were linked to lab colonies of mice maintained

in research institutes. Almost concurrently, it was shown that the whole mouse was not required for the maintenance of LCMV, but contact with cell cultures—particularly those of transplantable tumors—was sufficient to result in substantial outbreaks among scientists and other personnel working in research settings.

Within a few years, LCMV infection derived from lab and pet hamsters dwarfed the number of traditional recognized infections acquired from wild mice. The hamster, once not a usual suspect, supplanted the mouse as the major link to human infection and disease—transforming itself into "the usual suspect." Through natural maintenance among wild mice, the ongoing risk of laboratory infections, LCMV's association with hamsters—laboratory and pet—and the ongoing phenomenon of human-to-human transmission, the public health significance of LCM has an assured future.

SEOUL VIRUS (SEOV) IN *RATTUS NORVEGICUS*

There are numerous parallels in the history of Seoul virus (SEOV) recognition and discovery of host and disease consequences with that described for LCMV. The disease caused by SEOV is similar to, but less severe than and more rarely identified as, that caused by other members of the genus *Hantavirus*. The characterization of hantaviral disease as a unique clinical syndrome across Eurasia preceded the identification and culture of the agent by several decades.

The earliest modern clinical descriptions of hantaviral disease were from the undeclared Soviet-Japanese border conflict (Battles of Khalkhyn Gol) in 1939 (Ishii, 1942; Kitano, 1944a,b; Smorodintsev et al., 1944, 1959) (**Figure 3**). Epidemiologic investigations of outbreaks among combatants led both sides to conclude that a zoonotic virus was responsible. Although neither group isolated the agent, the Soviets identified the likely reservoir as a species of the genus *Myodes*, while the Japanese indicated that *Apodemus* was the likely source (Smorodintsev et al., 1959; Traub and Wisseman, 1978). The "rediscovery" of the syndrome occurred during the United Nations action in Korea during the early 1950s, when a disease with a matching presentation was reported among several thousand troops (Traub and Wisseman, 1978). As in prior studies, epidemiologic investigations suggested a rodent-borne zoonotic virus as the likely cause of what was referred to as "Korean hemorrhagic fever" (KHF). The means of virus transmission were not known at this time and some investigations centered around identifying a possible arthropod vector.

Roughly two decades later, Lee and colleagues (Lee et al., 1978) demonstrated viral antigen in the lungs of *Apodemus agrarius* using convalescent-stage human sera in an indirect immunofluorescent antibody assay (IFA). The subsequently isolated virus was named Hantaan virus after a river in South Korea, in keeping with place designations for proximate locations where zoonotic and arboviruses are first isolated.

Shortly after Lee's development of serological tests he identified a case of disease in an urban maintenance worker whose history was notable for having killed a rat (genus *Rattus*). *Apodemus* spp. are largely absent from most city environments and Lee, and colleagues focused on *R. norvegicus* and the black rat (*Rattus rattus*) and isolated a second Hantavirus, SEOV, from these species (Lee et al., 1982). This led to international concern that SEOV might have become or would become globally disseminated along with the introduced and cosmopolitan synanthropic *Rattus* spp. Studies to address these possibilities revealed the worst-case scenario; Norway rats infected with SEOV were found on nearly every continent (LeDuc et al., 1986). Spillover infections to other rodents were not observed and the genus *Rattus* was presumed to be the reservoir host (Korch et al., 1989). Hereafter we mainly focus on the Norway rat's role in the maintenance and transmission of SEOV.

CHARACTERISTICS OF HANTAVIRUSES

SEOV is a negative-sense single-stranded RNA virus that is tri-segmented (McAllister and Jonsson, 2014). The three gene segments are identified as L, M, and S. The S segment codes for the nucleocapsid protein, while the M segment codes for two glycoproteins (Gn and Gc). The L segment encodes the RNA-dependent RNA polymerase (McAllister and Jonsson, 2014). Hantaan virus represents the type species of the family *Bunyaviridae* while SEOV is likely among the most widely distributed member of the group. For an extended period of time, hantaviruses were presumed restricted to three major rodent groups. The exception was the description of amplification of Thottapalayam virus using RT-PCR against a targeted region in the S-segment of other hantaviruses (Arthur et al., 1992). The virus was isolated from the Asian house shrew (*Suncus murinus*)—an insectivore. More recently, identification of other hantaviruses from additional insectivore species and bats suggests a more complex and diverse characterization of this group (Klempa, 2018).

HUMAN INFECTION AND DISEASE

SEOV infection in humans presents as hemorrhagic nephrosonephritis or hemorrhagic fever with renal syndrome (HFRS; Smorodintsev et al., 1944). As with the other Old World hantaviruses, the name emphasized the hemorrhagic and renal aspects of infection, rather than the cardiac and pulmonary components of New World viruses, described in the early 1990s with the discovery of Sin Nombre virus (SNV) in the Southwestern United States (Nichol et al., 1993).

The course of severe HFRS has been divided into four stages; a prodromal with non-specific characteristics of fever, aches, chills, and often accompanied by headache; a hypotensive phase marked by thrombocytopenia and hypoxemia occurring 3–7 days after initial onset; a hypertensive stage with renal dysregulation and pronounced oliguria or anuria; and a convalescence phase with spontaneous return of renal function, often associated with polyuria and hypotension. Survivors often experience a protracted convalescence of weeks/months. Most deaths occur during phase three (Smadel, 1953; Traub and Wisseman, 1978).

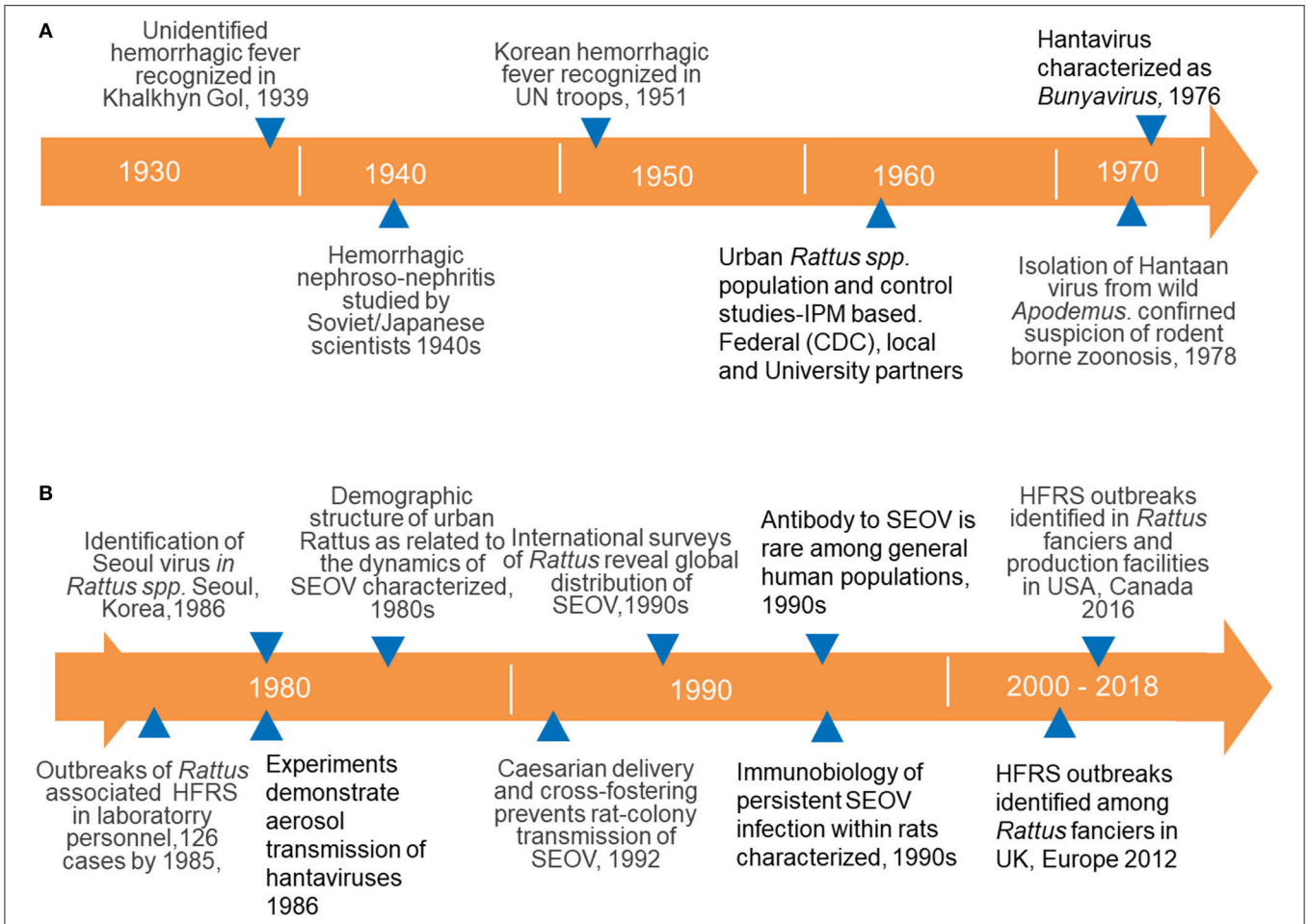

FIGURE 3 | (A) Time lines documenting the history of major events in our understanding of Old World hantaviruses causing HFRS, focusing on SEOV isolated from *Rattus* spp.; from the discovery of a new clinical entity (HFRS) to the identification of virus maintenance among rodents and the taxonomic designation of hantaviruses with the family Bunyaviridae; **(B)** details of our understanding of the epidemiology, maintenance, immunobiology, and transmission of SEOV to humans and the increase in recognized SEOV cases associated with ownership of pet rats.

DIAGNOSIS OF SEOV

Human immune responses to infection are marked (as with rats) with IgM and IgG antibody responses usually beginning 1–2 weeks after infection (Lee and Johnson, 1982). The presence of both IgM and IgG antibodies or a four-fold rise in IgG antibody titers are considered diagnostic of acute infection (LeDuc et al., 1990; Mattar et al., 2015). Detectable IgG persists for extended times (Mattar et al., 2015) so that IgG in the absence of IgM is considered evidence of previous infection.

Many of the early efforts for discovery and diagnosis of hantaviral infections used thin sections of fixed infected tissues (often lung) obtained from naturally infected wild rodents or from intentionally infected lab animals as the antigen for screening sera for antibodies. Application of a fluorescent-tagged secondary antibody resulted in a highly specific and sensitive test—the indirect immunofluorescent antibody assay, or IFA. These methods were used to diagnose human infections and to document seroconversion and antibody dynamics in experimentally infected rats and other species (Lee et al., 1978, 1982). With the successful isolation of Hantaan virus and its adaptation to tissue culture, infected Vero E-6 cells fixed to slides became the standard substrate for IFA tests (Lee et al., 1978). Later enzyme-linked immunosorbent assays (ELISA) and Western blots (and variants) were developed and used for screening and identifying important immunologically active proteins (Schmaljohn et al., 1990).

However, plaque reduction neutralization tests (PRNT) are considered the gold standard of serological tests as they measure the function rather than simply the binding of antibodies. PRNT also allows differentiation of various hantaviruses potentially causing the infection through comparison of end-point titers.

In the 1990s, reverse transcriptase polymerase chain reaction (RT-PCR) was first applied to the S segment of hantaviruses and demonstrated the utility of the technique for virus detection and differentiation by amplicon size (Arthur et al., 1992). This development allowed direct identification of SEOV and other hantaviral RNA in tissues. With the advent of sequencing

technology with RT-PCR, it was demonstrated that amplicon sequences varied sufficiently among humans and rodent reservoir hosts that fine scale heterogeneities could be used to determine geographic sites of infection. This ability was dramatically highlighted by studies elucidating the cause and epidemiology of hantavirus pulmonary syndrome (HPS) caused by *Peromyscus*-associated SNV in the Southwestern United States in 1993 (Nichol et al., 1993).

EPIDEMIOLOGY OF SEOV ASSOCIATED WITH WILD RATS

In humans, infection with SEOV reflects the extent to which individuals contact infectious rats, although transmission from wild rats is rare. This includes urban locations where high-density populations of humans living in low SES neighborhoods, lacking amenities such as routine garbage removal (**Figures 1A,C,D**), are frequently exposed to rats and the prevalence of SEOV infection among adult animals often exceeds 50% (see section below). As with other hantaviruses, risk of exposure and infection is defined by the characteristics of the human population, the environment features conducive to supporting rat populations and interactions capturing them in time and space. The usual route of SEOV transmission from wild rats to humans is presumed by aerosols of dried excreta/secreta.

Researchers speculate on drivers contributing to the rarity of diagnosed cases, but few studies have attempted to untangle the many possible factors. Although biological and/or environmental barriers presumably preclude ready transmission and infection of humans with SEOV, physician awareness of this rare disease is negligible and public health surveillance nonexistent. Acute illness caused by SEOV, like LCMV, does not have a unique presentation and signs and symptoms are consistent with a wide range of diseases (Glass et al., 1994). As with LCM, specialized testing laboratories are unavailable at most point-of-entry hospitals and ascribing etiologic cause to agents like these is a rarity.

The rarity of infection is exemplified by a serosurvey of more than 1,100 individuals visiting a STD clinic in inner city Baltimore, Maryland, United States, where only four individuals had antibodies to SEOV (diagnosed by IFA and confirmed by PRNT; **Figure 1D**; Childs and Glass, 1988; Childs et al., 1988a, 1991). During interviews, all four individuals (prior to knowing their exposure status) volunteered that part of their daily home maintenance was to sweep rat feces from the paved areas of their properties. Cleaning activities involving sweeping up rodent feces are a common risk factor for acquiring hantaviral infection, presumably due to large or small particle aerosols.

When focusing longitudinal serosurveys of special populations exhibiting signs suggestive of possible current or past SEOV infection (e.g., renal dysfunction measured by high levels of proteinuria or requiring dialysis), three acutely infected individuals were identified based on a four-fold rise in PRNT titers. Two of the three had primary symptoms consistent with HFRS, including striking renal insufficiency and shock (Glass et al., 1994). This suggests there is some background level of ongoing transmission by the usual routes to human populations from wild rats even in the absence of recognized threat.

SEOV PREVALENCE, MAINTENANCE, AND TRANSMISSION AMONG NORWAY RATS

Surveys for evidence of SEOV in wild Norway rats show substantial variation in prevalence. Even in the earliest studies (LeDuc et al., 1986), there was striking geographic variation. These surveys showed prevalence ranging from single digits, in various African cities, to 20–40% in Asian and Australian cities. In North America, most urban areas showed prevalence between these ranges, except for cities in California (United States), where infection was not reported, and Baltimore, where it reached the global extreme. This led to a series of studies in that city to better characterize the maintenance of the virus in the rodent host and suggested additional reasons why prevalence could appear to vary widely in wild rat populations.

One of the first observations was the non-monotonic change in antibody prevalence with age (size) in the rats. An initially high, but rapidly decreasing, prevalence of anti-SEOV antibody among the smallest wild-caught Norway rats was identified as due to maternal antibody (**Figure 4**). As maternally-derived antibodies waned, reaching a nadir just prior to the onset of sexual maturity, juvenile and sub-adult rats (based on sexual maturity and body mass determinations) were infected by SEOV through horizontal transmission routes (detailed below), producing a characteristic "J-shaped" distribution from cross-sectional sampling (**Figure 3**; Childs et al., 1987a, 1988b).

This phenomenon of maternal antibody-derived protection of neonates challenged with SEOV is reproducible in lab-reared rats, where immunity lasts for 4–6 weeks (Zhang et al., 1988). An additional finding corroborating the protective effects of maternally-derived IgG is the near absence of viral RNA detected by RT-PCR in rats (**Figure 4**). In larger/older rats, the prevalence of infection, as measured both by viral RNA detected by RT-PCR and serology, can increase until most rats show evidence of infection—∼80% in Baltimore, which appears to be a hyper-endemic area (LeDuc et al., 1986; Childs et al., 1987b; Glass et al., 1988).

SEOV infection in rats results in persistent infection, even in the presence of high levels of IFA and neutralizing antibodies. Initial isolation efforts are most often successful by targeting individuals with high antibody titers (Childs et al., 1987b) as opposed to LCMV, where horizontally infected mice clear the virus and seroconvert (see LCMV sections above). SEOV infection among wild rats causes no obvious overt disease and survivorship, fecundity, and fertility are unaffected among wild *R. norvegicus* (Lee et al., 1986; Childs et al., 1989).

In experimental systems, however, neonates born to uninfected dams and then infected with SEOV prior to seven days of age develop a progressive severe clinical course characterized by weight loss, ruffled fur, ataxia, limb paralysis, convulsions, and death, most often within 35 days (Zhang et al., 1988). These observations stand in stark contrast to the protective effect of maternal antibody in protecting neonates

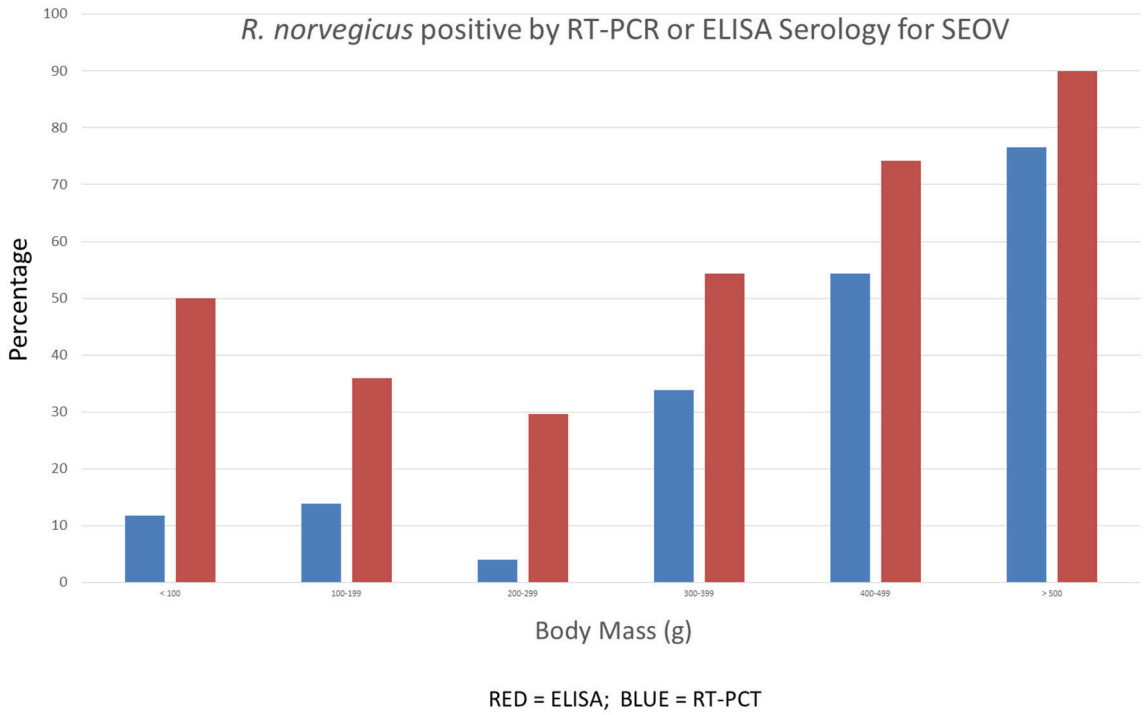

FIGURE 4 | Cross-sectional patterns of antibody and RT-PCR prevalence to SEOV among 485 wild Norway rats sampled from Baltimore, MD. The prevalence of antibody titers and RT-PCR prevalence decline among juvenile rats before rising steadily among adult rats (details are discussed in the text).

from pathogenic effects (Zhang et al., 1988; Dohmae and Nishimune, 1995).

IMMUNOBIOLOGY OF SEOV PERSISTENCE IN NORWAY RATS

The existing dogma states that hantaviral infection alone did not cause overt disease in rats (or other reservoir hosts for different hantaviruses) except under experimental conditions described above (Yanagihara et al., 1985; Lee et al., 1986; Hutchinson et al., 1998; Botten et al., 2000; Wahl-Jensen et al., 2007; Eyzaguirre et al., 2008). How rats and other rodents infected with hantaviruses avoid the deleterious effects are not well understood. Recently, advances have been made in elucidating the basis for the persistence of SEOV within rats in the face of robust humoral immune responses.

Persistence is driven by the upregulation of regulatory responses and downregulation of proinflammatory responses. SEOV preferentially replicates in rat lungs (Khaiboullina and St. Jeor, 2002), where the virus is found both in alveolar macrophages (AMs) and endothelial cells. This may polarize CD4+ T cell differentiation toward a regulatory T (Treg) cell phenotype. *In vitro*, viral infection increases immunological tolerance by promoting transforming growth factor beta (*Tgfβ)* mRNA in AMs and programmed cell death 1 ligand 1 (PD-L1) in lung microvascular endothelial cells (LMVECs). SEOV-infected LMVECs, but not AMs, induce increased expression of *Foxp3* expression (the transcriptional factor in Treg cells) and Treg cell frequency in allogeneic CD4+ T cells (Li and Klein, 2012). *In vivo*, elevated regulatory responses include TGF-β1 and numbers of CD4+CD25+FoxP3+ Treg cells in the lungs. This is associated with persistence of SEOV (Easterbrook et al., 2007a; Schountz et al., 2007; Easterbrook and Klein, 2008a).

In addition to the regulatory responses, activity along the type I interferon (IFN) pathway and proinflammatory cytokines (e.g., IL-1β, IL-6, TNF-α, and IFN-γ) remain at or below baseline throughout SEOV infection in the lungs of male, but not female, Norway rats (Klein et al., 2004a; Easterbrook and Klein, 2008a; Hannah et al., 2008). Conversely, in the spleen, proinflammatory, and antiviral responses are elevated during acute SEOV infection in both sexes (Easterbrook and Klein, 2008a). Infection of bone marrow-derived macrophages (BMDMs) with SEOV suppresses NF-κB-mediated inflammatory responses, including TNF-α, IL-6, and IL-10, and surface marker expression (i.e., MHCII, CD80, and CD86), suggesting SEOV infection suppresses the innate immune response in antigen-presenting cells (Au et al., 2010).

These pathways suggest a reason for the observed sex difference in infection prevalence, with males having a higher likelihood of infection than females (Glass et al., 1998; Bernshtein et al., 1999; Hinson et al., 2004; Klein et al., 2004b); (Douglass et al., 2007). Males have more SEOV RNA and antigen in target organs and saliva (i.e., vehicle for virus shedding) than do females (Klein et al., 2001; Hannah et al., 2008). In the laboratory, removal of the testes in males to reduce androgen levels also reduces SEOV loads, whereas removal of the ovaries

in females increases SEOV RNA loads (Hannah et al., 2008). The expression of innate antiviral (e.g., *Tlr7, Myd88*, *Rig-I*, *Visa*, *Ifnβ*, and *Mx2*) and proinflammatory (e.g., *Tnfα* and *Ccl5*) factors is higher in the lungs of gonad intact females rats than in males (Klein et al., 2002, 2004a; Easterbrook and Klein, 2008b; Hannah et al., 2008), suggesting that this contributes to reduced viral loads in females compared to males. Conversely, the expression and production of regulatory factors, including *Foxp3* and TGF-β, are elevated in the lungs of males compared to females (Easterbrook and Klein, 2008b). This suggests that sex differences in immune responses during hantavirus infection may be driven by estradiol in females and testosterone in males, as gonadectomy reverses these differences (Klein et al., 2004a; Hannah et al., 2008). It is plausible that reduced innate and proinflammatory defenses and elevated regulatory responses combined with an increased propensity to engage in aggression may contribute to increased maintenance and transmission of hantaviruses among male as opposed to among female rodents.

THE USUAL AND UNUSUAL ROUTES OF HUMAN EXPOSURE TO SEOV

Human exposure to SEOV requires contact with infectious materials from *R. norvegicus*. As the virus is excreted in feces, urine and secreted in saliva (Klein et al., 2000), direct contact with the animal itself is not needed. The first documentation of hantavirus transmission by the indirect aerosol route was described among Soviet laboratory workers, facility cleaners and secretaries who were infected, often without any near contact with a colony of *A. agrarius* (Nuzum et al., 1988).

The low prevalence of antibodies among urban residents with putatively high levels of exposure to infected rats indicates there is a significant barrier to cross-species transmission. Whether this barrier(s) is driven by cultural, social, biological, or environmental factors (or more likely a combination of these) is not known.

ENTER LABORATORY AND PET RATS

A twist in the risk of SEOV exposure involved a category of interactions with *R. norvegicus* where the intent of humans is to interact with the animals themselves (**Figure 3B**). As with LCMV, these at-risk groups are exposed to potentially infectious animals through occupation (research settings) or avocation (pet owners). These interactions with rats are outside the realm of usual interactions with wild Norway rats.

The risk of human exposure and infection among laboratory researchers and individuals working in breeding facilities with Norway rats was foreshadowed by the outbreaks among research labs housing *Apodemus* spp. (detailed above). HFRS outbreaks associated with SEOV first occurred among personnel working with lab rats in Korea and Japan (of note, these occurrences also provided some additional evidence that an arthropod vector was not necessary for SEOV transmission) (Umenai et al., 1979; Lee and Johnson, 1982; Kawamata et al., 1987). Subsequent outbreaks of SEOV-associated disease among laboratory staff in Europe (Desmyter et al., 1983; Lloyd et al., 1984) confirmed that unapparent infections among laboratory stocks of *Rattus* were not isolated events and posed a significant health threat, and remedial actions were instituted. One important step to prevent maintenance of SEOV in the laboratory was to adopt caesarian delivery and cross-fostering of pups to known uninfected dams (**Figure 3B**) (McKenna et al., 1992).

In parallel with the unfolding history of LCMV, SEOV was also identified in hybridomas of rat tissues in research laboratories (**Figures 2A**, **3A**). Once again, it became apparent that the intact reservoir rodent was not required for viral persistence. Upon retrospective analyses, cell lines were shown to remain persistently infected in excess of 8–10 years, indicating the past and ongoing health risk to laboratory personnel and the specter of contamination of other cell lines, as occurred when LCMV was present in cell cultures producing rabies vaccine (see LCMV sections above) (Lloyd and Jones, 1986). Once serological diagnostic tests became commonplace, extensive infection among cell lines was eliminated by destroying contaminated cell lines and culling infected rats in research and commercial settings to reduce recurrent reinfection.

Although contamination and productive infection by SEOV, LCMV and other zoonotic viruses (e.g., SV40) is now a well characterized and largely controllable phenomenon among lab animals and cell lines, the next twist to SEOV transmission to humans was unanticipated and posed new challenges to control. The first reports that owners and breeders of fancy rats were infected were published in 2013 from Europe and several localized outbreaks were identified (**Figure 3B**) (Jameson et al., 2013; Lundkvist et al., 2013; McElhinney et al., 2017; Reynes et al., 2017). Similar findings from the USA followed (**Figure 3B**).

The association between pet rats and human SEOV was only recognized by the occurrence of HFRS in humans rather than by any screening of rats destined for the pet trade. Serological and virological surveys during investigations of human cases found levels of SEOV to be between 80 and 100% among breeding colonies—a figure higher than the prevalence found among wild rat populations! As these colonies were identified after the human cases this does not, presumably, reflect the typical prevalence among breeding colonies more generally. However, the extent of human infection dwarfed the previous number of acute SEOV cases resulting from exposures to wild or laboratory animals. Surveys of more than 800 people involved in *Rattus* work, either professionally or for avocation, found evidence of SEOV infection in more than a third of this population (Duggan et al., 2017). The highest prevalence was among persons maintaining rats as pets, with substantially lower rates in those involved with professional activities in laboratories. Considering that the prevalence of SEOV antibodies is <0.5% among populations of urban residents living in conditions where dense populations of Norway rats exist and where a large proportion are infected by SEOV (**Figures 1C,D**), the results reported from surveys of rat owners were staggering. When it was considered as dogma to accept that SEOV transmission to humans was rare, these results indicated that in the right settings, transmission was actually common.

Most recently, a sizable outbreak of SEOV was identified among pet rat owners in North America (Kerins et al., 2018). The disease was identified in two individuals and follow-up studies showed that more than 10% of people tested were antibody-positive to SEOV. Investigations of more than 100 facilities found evidence of infection either in people or *Rattus* at more than 30% of sites. These findings—shifting attention from the usual (albeit rare) epidemiologic cycle of SEOV transmission from wild rat to human, or from the more unusual laboratory rat or cell line to human—demonstrated that ownership and interactions with pet rats changed our whole notion about risks for infection.

Two major findings emerged from these outbreaks among pet owners. Of public health concern was the number of SEOV cases and severity of disease among rat owners. The numbers of humans involved eclipsed the few sporadic cases of disease/infection reported among the general public interacting with the wild reservoir of SEOV—an eerie parallel to the emergence of LCMV among owners of hamsters. Secondly, the introduction of SEOV into rat breeding colonies, aimed for the fancy rat admirer or simply persons wanting a plain rat pet, was geographically extensive and was likely to be found beyond Europe and North America. An immediate consequence of these reports was that no adequate surveillance and control policies were in place to protect the public, as breeding of rats destined for the pet trade does not require a dedicated facility but can be a household or cottage industry; control will be challenging and incomplete at best. This represents déjà vu for persons previously following LCMV's peregrinations.

The likely reason for these high rates of infection among rat fanciers has been explored in Europe (Robin et al., 2017). In semi-structured surveys it was concluded that owners re-framed the perception of their rats to "pets" away from the perspective of synanthropic wild animals, so that the fancy rat became qualitatively distinct and was presumed "clean"—in essence a "new" species divorced from pestilential baggage carried by the wild ancestor. In the absence of overt clinical disease in the pet rat and lack of awareness of how persistent SEOV infection can perpetuate infection from generation to generation, there was simply a misperception among owners of the potential risk of rat-to-human transmission. This was one proposed reason for why levels of SEOV infection among owners of fancy rats were elevated nearly 20-fold above that of commercial breeders (Duggan et al., 2017).

CONTROLLING SEOV TRANSMISSION

The traditional approach to reduce SEOV exposure and transmission from wild Norway rats involves rat population control in urban areas. Population reduction is achievable but eradication of rats even from intensely targeted locations is rarely achieved, and populations typically rebound over months even when reductions may exceed 50% (Gardner-Santana et al., 2009; Glass et al., 2016). Control efforts have typically relied heavily on the application of rodenticide, and still do in most urban settings, but the implementation of integrative pest management (IPM) has achieved longer dampening of populations as they can alter the carrying capacity of targeted locations (Keiner, 2005).

Early attempts to mitigate populations through IPM involved both ecological and environmental interventions to reduce carrying capacity by reducing food and shelter resources, with complementary population reduction by trapping or chemical means (see Davis, 1953). The IPM approach was embraced by CDC initiatives in the 1970s directed at improving conditions to reduce rat infestation levels through a block-by-block intervention protocol carried out within targeted cities (including Baltimore), which was effective but not sustainable (Centers for Disease Control and Prevention, 1981, 1982a,b).

As a rare case in point, Baltimore reinstated these protocols in the mid-1990s in response to rat infestations in several city locations that proved resistant to rodenticide applications. A combination of targeted rodenticide-baiting, trash collection, public education, reduction in nesting sites and other habitat modifications were applied in two neighborhoods of ∼1,700 households (Lambpropolous et al., 1999). One of the neighborhoods was heavily infested (21% households with rats in adjoining alleys or within homes), while in the other rat infestation was only 4%. After a 3-month intervention, infestations had been reduced to 8 and 0.3%, respectively. At 6 months post-intervention, infestation levels in the heavily infested neighborhood had returned to 20% while rat populations were undetectable in the less intensively infested area. Based on the limited sample size, the conclusion was that there may be some level of infestation above which ongoing IPM control efforts will fail to solve effectively in the long term.

The cause of this IPM failure was the basis for further studies. As observed repeatedly (Abelkrim et al., 2005; Gardner-Santana et al., 2009; Combs et al., 2018), Norway rats show highly structured populations with substantial population/genetic isolation by distance over hundreds of meters. Examination of maternity/paternity dyads in wild rats showed, however, distinct mate-seeking behavior compared to normal movements. While most non-sexual activities among individual rats occur within 100 m (Glass et al., 1989, 2016), mates often came from more distant alleys (likely beyond the geographic range of the IPM studies), essentially expanding the potential pool of mates and buffering local populations from perturbations.

Consequently, new strategies are needed to target interconnected populations. Population genetic studies that trace and link rat-infested locations by determining levels of gene flow should help to inform intervention efforts by defining and targeting an Eradication Unit rather than relying on block-by-block or simple community-based control (Robertson and Gemmell, 2004).

Although the outcomes of single or limited interventions to control targeted rat populations may be unconvincing, the conclusion that sustained IPM strategies are critical is unchanged. We have no other actionable options that simultaneously address human and rat behavior at the community level, mitigate environmental conditions that provide key resources for rat populations and improve sanitation (garbage and major refuse removal) measures provided by local government agencies.

However, examples of long-term community and political commitment to sustain efforts are few and rarely achieved (Childs and Glass, 1988; Lambpropolous et al., 1999).

Reduction of SEOV infection of lab colonies of rats has been achieved in part by placing the burden of screening and culling on commercial operations maintaining breeding stock. However, ongoing surveillance of such colonies is required as ongoing reintroduction of SEOV (and other pathogens) from wild rats remains a threat (Easterbrook et al., 2007b; Reynes et al., 2017).

Individuals who place themselves at risk of SEOV infection by intentionally adopting rats as pets pose a unique and challenging group for intervention. As noted by Robin et al. (2017), this group has a perception that their domesticated rats pose little or no risk. Prevention for this group is more direct but has practical challenges influenced by the rat–virus interactions that produce persistent, unapparent infections in the reservoir. Historically, most pest control strategies focused on the inadvertent exposure population rather than this group. In the absence of routinely scheduled diagnostic testing of breeding colonies, which range from commercial enterprises to cottage industries, the lack of apparent disease among pet rats will permit persistent infection among closely housed individuals and across generations.

SUMMARY AND CONCLUSIONS

LCMV and SEOV are rodent-borne zoonotic pathogens that enjoy a global or near global distribution. The murid rodents, *M. musculus* and *R. norvegicus*, were introduced with ship-borne commerce and colonization and are the primary reservoir hosts for LCMV and SEOV, respectively.

The original concept of the zoonotic cycle linking transmission of LCMV and SEOV to humans through contact with their wild rodent hosts was established within a year after the discovery of LCMV as a cause of aseptic meningitis but required several decades to definitively link wild rodents as the source of viruses causing HFRS among humans (**Figures 2**, **3**). A few years after the isolation of HTNV, the closely related SEOV was isolated from urban *Rattus* spp. and was shown to be responsible for HFRS among residents of Seoul, Korea. In this way, the natural or usual zoonotic transmission cycle was established linking human disease to wild rodent contact or proximity.

Within years, subsequent reports of laboratory-acquired infections caused by both viruses indicated that breeding stocks of rodents were contaminated. Patterns of infection and disease implicated aerosol transmission of LCMV and SEOV shed within rodent secreta/excreta, indicating that physical contact with the reservoir host was not required, adding an important element into the simple zoonotic cycle. It was also the first indication that simple zoonoses of wild animals had slipped unrecognized into human enterprises on a broader scale than had been previously appreciated. The realization that breeding stocks of rodents were infected with both LCMV and SEOV and were the source of human disease was not anticipated and raised questions about health security among lab personnel, commercial breeders and, importantly, the reliability of scientific studies using potentially infected rodents as the basis for experimental outcomes.

Almost concurrently it was shown that the whole mouse or rat was not required for the maintenance and transmission of LCMV or SEOV, but contact with virus-contaminated cell cultures, particularly those of transplantable tumors or hybridomas, was sufficient to produce substantial outbreaks among scientists and other personnel working in research settings. The usual and traditional zoonotic cycle of virus maintenance among wild rodents and transmission to humans via aerosolization of, or contact with, infectious secreta/excreta was far too simple. The transmission from inadvertently infected conspecific laboratory animals was deemed unusual but understandable, but infection from cell cultures, often repeatedly passed, was out of the ordinary and an unusual twist to be added within the expanding concept of the zoonotic cycle. Of particular note, outbreaks of LCMV- and SEOV-related infection/disease were more extensive than reported in natural settings, where only the simplest cycle existed.

Within years of the discovery of lab mice and mouse-derived cell cultures as the source of human disease, a nearly identical pattern of discoveries unfolded—but in these instances the source of LCMV was from hamsters (most notably the golden hamster), a different rodent species in a taxonomically removed family. In short order, infections were being diagnosed within research settings and among residents keeping golden hamsters as pets. Outbreaks caused by LCMV among hamster owners exceeded by several orders of magnitude the total number of acute cases ascribed to contact with wild house mice—the original and only species in the initial scheme of the zoonotic cycle. Not only had a distantly related rodent become involved in what had become the most "routine" transmission of LCMV to humans, its public health significance as the most important intermediary in the zoonotic cycle of transmission to humans established this species as the new reigning reservoir host. This unusual twist completely rearranged our thinking on zoonotic cycling of LCMV.

In a manner that partially mirrors events driving our new understanding of LCMV epidemiology, with SEOV, which rarely causes clinically diagnosed infection among the general population that has high levels of exposures to infected wild Norway rat populations, there has emerged disease outbreaks among persons owning fancy rats as pets. Like owning pet hamsters, these are inadvertent but intentional induced exposures from animals assumed to be virus free. Controlling these unusual routes of transmission are daunting and requires efforts entirely different from routine IPM measures aimed at controlling wild rodent populations. In both LCMV and SEOV, the immunobiology of rodent–virus interactions produces occult infections that require substantial diagnostic efforts to eliminate the threat.

At this juncture, the different, unusual, and unpredicted routes of LCMV transmission diverge from that of SEOV. Beginning with the demonstration that human-to-human transmission from acutely infected women during pregnancy resulted in severe, often fatal consequences to the fetus and permanent sequelae among surviving offspring (**Figure 2B**),

a new twist, again unanticipated, was identified. Human-to-human transmission of LCMV to solid organ recipients caused severe, and most often fatal, disease (**Table 1**). In some cases, infection among donors was linked to ownership of pet hamsters while in most cases the source of infection remained undetermined. Traceback studies showed that infected breeding stocks distributed to wholesale suppliers were the source of infection to pet owners. Of note, these rare occurrences highlighted how often LCMV infection goes unsuspected and undiagnosed.

The evolution of transmission routes for human infection by LCMV and SEOV have unfolded the highly complex interactions, deviating dramatically from the usual or classically defined zoonotic cycle of one reservoir species transmitting these viruses directly or indirectly to humans. A bewildering transmission web has evolved, linking the interaction of multiple variables to human infection, as humans have found new ways to interact with these rodents.

One fact is the maintenance of both viruses within wild rodents will continue to provide renewed sources for virus spillover to other rodent hosts owned as pets. These viruses have surprised us on numerous occasions, and whether no new string will be added to the complex web of transmission is uncertain. However, the epidemiology of these viruses' urban infections promotes that possibility. The global percentage of humans living in cities has surpassed 50% and continues to grow. Inevitably, many residents of burgeoning cities will live in conditions highly conducive to significant human–rodent interactions and these will be locations for examining increased incidence/prevalence of human infection and disease. Whatever twists remain, these viruses are here to stay and their significance in causing substantial public health challenges, perhaps limited today, is likely to increase.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

Abelkrim, J., Pascal, M., Calmet, C., and Samadi, S. (2005). Importance of assessing population genetic structure before eradication of invasive species: examples from insular Norway rat populations. *Conserv. Biol.* 19, 1509–1518. doi: 10.1111/j.1523-1739.2005.00206.x

Ackermann, R., Bloedhorn, H., Küpper, B., Winkens, I., and Scheid, W. (1964). Über die verbreitung des virus der lymphocytären choriomeningitis unter den Mäusen in Westdeutschland. *Zen. Bakter. Parasit. Infekt. Hyg.* 194, 407–430.

Ackermann, R., Stille, W., Blumenthal, W., Helm, E. B., Keller, K., and Baldus, O. (1972). Syrische goldhamster als uberträger von lymphozytärer choriomeningitis. *Deut. Med. Wochen.* 97, 1725–1731. doi: 10.1055/s-0028-1107638

Adler, S. (1948). Origin of the golden hamster cricetus auratus as a laboratory animal. *Nature* 162:256. doi: 10.1038/162256b0

Ahmed, R., Hahn, C. S., Somasundaram, T., Villarete, L., Matloubian, M., and Strauss, J. H. (1991). Molecular basis of organ-specific selection of viral variants during chronic infection. *J. Virol.* 65, 4242–4247.

Albariño, C., Palacios, G., Khristova, G., Erickson, M. L., Carroll, B. R., Comer, S. A., et al. (2010). High diversity and ancient common ancestry of Lymphocytic Choriomeningitis virus. *Emerg. Infect. Dis.* 16, 1093–1100. doi: 10.3201/eid1607.091902

American Veterinary Medical Association (2012). *U.S. Pet Ownership & Demographics Sourcebook*. Schaumburg, IL.

Amman, B. R., Pavlin, B. I., Albarino, C. G., Comer, J. A., Erickson, B. R., Oliver, J. B., et al. (2007). Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. *Emerg. Infect. Dis.* 13, 719–725. doi: 10.3201/eid1305.061269

Armstrong, C., and Sweet, L. K. (1939). Lymphocytic choriomeningitis. *Public Health Rep.* 54, 673–684. doi: 10.2307/4582864

Armstrong, C. L., and Lillie, R. D. (1934). Experimental Lymphocytic Choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St. Louis Encephalitis Epidemic. *Public Health Rep.* 49, 1019–1020. doi: 10.2307/4581290

Armstrong, D. F., Fortner, J. G., Rowe, W. P., and Parker, J. C. (1969). Meningitis due to Lymphocytic Choriomeningitis virus endemic in a hamster colony. *JAMA* 209, 465–467. doi: 10.1001/jama.1969.03160150051019

Arthur, R. R., Lofts, R., Gomez, J., Glass, G. E., LeDuc, J. W., and Childs, J. E. (1992). Grouping of hantaviruses by S genome segment PCR and amplification of viral RNA from wild-caught rats. *Am. J. Trop. Med. Hyg.* 46, 410–424.

Asnis, D. S., Muana, O., Kim, D. G., Garcia, M., Rollin, P. E., and Slavinski, S. (2010). Lymphocytic Choriomeningitis virus meningitis, New York, NY, USA, 2009. *Emerg. Infect. Dis.* 16, 328–330. doi: 10.3201/eid1602.091347

Au, R. Y., Jedlicka, A. E., Li, W., Pekosz, A., and Klein, S. L. (2010). Seoul virus suppresses NF-kappaB-mediated inflammatory responses of antigen presenting cells from Norway rats. *Virology* 400, 115–127 doi: 10.1016/j.virol.2010.01.027

Barber, D. L., Wherry, E. J., Masopust, D., Zhu, B., Allison, J. P., Sharpe, A. H., (2006). Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439, 682–687. doi: 10.1038/nature04444

Barton, L. L. (2010). Human infection with Lymphocytic Choriomeningitis virus. *Emerg. Infect. Dis.* 16, 1046–1047. doi: 10.3201/eid1606.100250

Barton, L. L., and Hyndman, N. J. (2000). Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. *Pediatrics* 105:E35. doi: 10.1542/peds.105.3.e35

Barton, L. L., Mets, M. B., and Beauchamp, C. L. (2002). Lymphocytic choriomeningitis virus: emerging fetal teratogen. *Am. J. Obstet. Gynecol.* 187, 1715–1716. doi: 10.1067/mob.2002.126297

Barton, L. L., Peters, C. J., and Ksiazek, T. G. (1995). Lymphocytic choriomeningitis virus: an unrecognized teratogenic pathogen. *Emerg. Infect. Dis.* 1, 152–153. doi: 10.3201/eid0104.950410

Becker, S. D., Bennett, M., Stewart, J. P., and Hurst, J. L. (2007). Serological survey of virus infection among wild house mice (*Mus domesticus*) in the UK. *Lab. Anim.* 41, 229–238. doi: 10.1258/002367707780378203

Benda, R. (1964). Passive immunoprophylaxis and immunotherapy of inhalation lymphocytic choriomeningitis in guinea-pigs. *J. Hyg. Epidemiol. Microbiol. Immunol.* 8, 243–251.

Bernshtein, A. D., Apekina, N. S., Mikhailova, T. V., Myasnikov, Y. A., Khlyap, L. A., Korotkov, Y. S., et al. (1999). Dynamics of Puumala hantavirus infection in naturally infected bank voles (*Clethrinomys glareolus*). *Arch. Virol.* 144, 2415–2428. doi: 10.1007/s007050050654

Biggar, R. J., Douglas, R. G., and Hotchin, J. (1975a). Letter: Lymphocytic choriomeningitis associated with hamsters. *Lancet* 1, 856–857. doi: 10.1016/S0140-6736(75)93027-5

Biggar, R. J., Schmidt, T. J., and Woodall, J. P. (1977). Lymphocytic choriomeningitis in laboratory personnel exposed to hamsters inadvertently infected with LCM virus. *J. Am. Vet. Med. Assoc.* 171, 829–832.

Biggar, R. J., Woodall, J. P., Walter, P. D., and Haughie, G. E. (1975b). Lymphocytic choriomeningitis outbreak associated with pet hamsters: fifty-seven cases from New York state. *JAMA* 232, 494–500. doi: 10.1001/jama.1975.03250050016009

Bishop, D. H., and Auperin, D. D. (1987). Arenavirus gene structure and organization. *Curr. Top. Microbiol. Immunol.* 133, 5–17. doi: 10.1007/978-3-642-71683-6_2

Bonthius, D. J., and Perlman, S. (2007). Congenital viral infections of the brain: lessons learned from lymphocytic choriomeningitis virus in the neonatal rat. *PLoS Pathog.* 3:e149. doi: 10.1371/journal.ppat.0030149

Botten, J., Mirowsky, K., Kusewitt, D., Bharadwaj, M., Yee, J., Ricci, R., et al. (2000). Experimental infection model for Sin Nombre hantavirus in the deer mouse *(Peromyscus maniculatus). Proc. Natl. Acad. Sci. U.S.A.* 97, 10578–10583. doi: 10.1073/pnas.180197197

Brooks, D. G., Trifilo, M. J., Edelmann, K. H., Teyton, L., McGavern, D. B., and Oldstone, M. B. (2006). Interleukin-10 determines viral clearance or persistence *in vivo*. *Nat. Med.* 12, 1301–1309. doi: 10.1038/nm1492

Buchmeier, M. J., Welsh, R. M., Dutko, F. J., and Oldstone, M. B. A. (1980). "The virology and immunobiology of Lymphocytic Choriomeningitis Virus infection," in *Advances in Immunology*, eds F. J. Dixon and H. G. Kunkel (Boston, MA: Academic Press), 275–331.

Centers for Disease Control and Prevention (1981). Urban rat control–United States. *MMWR Morb. Mortal. Wkly. Rep.* 30:205.

Centers for Disease Control and Prevention (1982a). Urban rat control–United States, second quarter, fiscal year 1982. *MMWR Morb. Mortal. Wkly Rep.* 31:602.

Centers for Disease Control and Prevention (1982b). Urban rat control–United States. *MMWR Morb. Mortal. Wkly. Rep.* 31:259.

Centers for Disease Control and Prevention (2006). Survey of lymphocytic choriomeningitis virus diagnosis and testing–Connecticut, 2005. *MMWR Morb. Mortal. Wkly. Rep.* 55:398.

Centers for Disease Control and Prevention (2008). Brief report: lymphocytic choriomeningitis virus transmitted through solid organ transplantation–Massachusetts, 2008. *MMWR Morb. Mortal. Wkly.* 57, 799–801.

Centers for Disease Control and Prevention (2014). A cluster of lymphocytic choriomeningitis virus infections transmitted through organ transplantation-Iowa, 2013. *MMWR Morb. Mortal. Wkly.* 63:249.

Centers for Disease Control and Prevention (2005). Lymphocytic choriomeningitis virus infection in organ transplant recipients–Massachusetts, Rhode Island, 2005. *MMWR Morb. Mortal. Wkly. Rep.* 54, 537–539.

Charrel, R. N., and de Lamballerie, X. (2010). Zoonotic aspects of arenavirus infections. *Vet. Microbiol.* 140, 213–220. doi: 10.1016/j.vetmic.2009.08.027

Childs, J. E. (2004). Zoonotic viruses of wildlife: hither from yon. *Arch. Virol. Suppl.* 18, 1–11. doi: 10.1007/978-3-7091-0572-6_1

Childs, J. E., and Glass, G. E. (1988). *Epidemiology of Hantavirus Infections in Baltimore*. USMRDC Contract No. DAMD17-87-C-7101.

Childs, J. E., Glass, G. E., Korch, G. W., Arthur, R. R., Shah, K. V., LeDuc, J. W., et al. (1988a). Evidence of human infection with a rat associated *Hantavirus* in Baltimore, Maryland. *Am. J. Epidemiol.* 127, 875–878. doi: 10.1093/oxfordjournals.aje.a114871

Childs, J. E., Glass, G. E., Korch, G. W., Ksiazek, T. G., and LeDuc, J. W. (1992). Lymphocytic choriomeningitis virus infection and house mouse (*Mus musculus*) distribution in urban Baltimore. *Am. J. Trop. Med. Hyg.* 47, 27–34. doi: 10.4269/ajtmh.1992.47.27

Childs, J. E., Glass, G. E., Korch, G. W., and LeDuc, J. W. (1987a). Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. *Am. J. Trop. Med. Hyg.* 37, 648–662. doi: 10.4269/ajtmh.1987.37.648

Childs, J. E., Glass, G. E., Korch, G. W., and LeDuc, J. W. (1988b). The ecology and epizootiology of hantaviral infections in small mammal communities of Baltimore: a review and synthesis. *Bull. Soc. Vector Ecol.* 13, 1–9.

Childs, J. E., Glass, G. E., Ksiazek, T. G., Rossi, C. A., Barrera Oro, J. G., and LeDuc, J. W. (1991). Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population of Baltimore. *Am. J. Trop. Med. Hyg.* 44:117121. doi: 10.4269/ajtmh.1991.44.117

Childs, J. E., Glass, G. E. G. W., Korch, G. W., and LeDuc, J. W. (1989). Effects of hantaviral infection on survival, growth and fertility in wild rat *(Rattus norvegicus)* populations of Baltimore, Maryland. *J. Wldlf. Dis.* 25, 469–476. doi: 10.7589/0090-3558-25.4.469

Childs, J. E., Korch, G. W., Glass, G. E., Shah, K. V., and LeDuc, J. W. (1987b). Epizootiology of *Hantavirus* infections in Baltimore: isolation of a virus from wild rats, and characteristics of infected rat populations. *Am. J. Epidemiol.* 126, 55–68. doi: 10.1093/oxfordjournals.aje.a114662

Childs, J. E., and Peters, C. J. (1993). "Ecology and epidemiology of arenaviruses and their hosts," in *The Arenaviridae,* ed M. S. Salvato (New York, NY: Plenum Press), 331–384. doi: 10.1007/978-1-4615-3028-2_19

Childs, J. E., and Wilson, L. J. (1994). "Lymphocytic choriomeningitis," in *Handbook of Zoonoses: Viral,* ed G. W. Beran (Boca Raton, FL: CRC Press), 483–491.

Combs, M., Puckett, E. E., Richardson, J., Mims, D., and Munshi-South, J. (2018). Spatial population genomics of the brown rat (*Rattus norvegicus*) in New York City. *Mol. Ecol.* 27, 83–98. doi: 10.1111/mec.14437

Davis, D. E. (1953). The characteristics of rat populations. *Quart. Rev. Biol.* 28, 373–401. doi: 10.1086/399860

Desmyter, J., LeDuc, J. W., Johnson, K. M., Brasseur, F., Deckers, C., and van Ypersele, S. C. (1983). Laboratory rat associated outbreak of haemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. *Lancet* 2, 1445–1448. doi: 10.1016/S0140-6736(83)90797-3

Dohmae, K., and Nishimune, Y. (1995). Protection against hantavirus infection by dam's immunity transferred vertically to neonates. *Arch. Virol.* 140, 165–172.

Douglass, R. J., Calisher, C. H., Wagoner, K. D., and Mills, J. N. (2007). Sin Nombre virus infection of deer mice in Montana: characteristics of newly infected mice, incidence, and temporal pattern of infection. *J. Wildl. Dis.* 43, 12–22. doi: 10.7589/0090-3558-43.1.12

Duggan, J. M., Close, R., McCann, L., Wright, D., Keys, M., McCarthy, N., et al. (2017). A seroprevalence study to determine the frequency of Hantavirus infection in people exposed to wild and pet fancy rats in England. *Epidemiol. Infect.* 145, 2458–2465. doi: 10.1017/S0950268817001480

Dykewicz, C. A., Dato, V. M., Fisher-Hoch, S. P., Howarth, M. V., Perez-Oronoz, G. I., Ostroff, S. M., et al. (1992). Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA* 267, 1349–1353. doi: 10.1001/jama.1992.03480100055030

Easterbrook, J. D., Kaplan, J. B., Vanasco, N. B., Reeves, W. K., Purcell, R. H., Kosoy, M. Y., et al. (2007b). A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol. Infect.* 135, 1192–1199. doi: 10.1017/S0950268806007746

Easterbrook, J. D., and Klein, S. L. (2008a). Seoul virus enhances regulatory and reduces proinflammatory responses in male Norway rats. *J. Med. Virol.* 80, 1308–1318. doi: 10.1002/jmv.21213

Easterbrook, J. D., and Klein, S. L. (2008b). Corticosteroids modulate Seoul virus infection, regulatory T-cell responses and matrix metalloprotease 9 expression in male, but not female, Norway rats. *J. Gen. Virol.* 89(Pt 11), 2723–2730. doi: 10.1099/vir.0.2008/03715-0

Easterbrook, J. D., Zink, M. C., and Klein, S. L. (2007a). Regulatory T cells enhance persistence of the zoonotic pathogen Seoul virus in its reservoir host. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15502–15507. doi: 10.1073/pnas.0707453104

Ejrnaes, M., Filippi, C. M., Martinic, M. M., Ling, E. M., Togher, L. M., Crotty, S., et al. (2006). Resolution of a chronic viral infection after interleukin-10 receptor blockade. *J. Exp. Med.* 203, 2461–2472. doi: 10.1084/jem.20061462

Emonet, S., Retornaz, K., Gonzalez, J. P., de Lamballerie, X., and Charrel, R. N. (2007). Mouse-to-human transmission of variant lymphocytic choriomeningitis virus. *Emerg. Infect. Dis.* 13, 472–475. doi: 10.3201/eid1303.061141

Eyzaguirre, E. J., Milazzo, M. L., Koster, F. T., and Fulhorst, C. F. (2008). Choclo virus infection in the Syrian golden hamster. *Am. J. Trop. Med. Hyg.* 78, 669–674. doi: 10.4269/ajtmh.2008.78.669

Farmer, T. W., and Janeway, C. A. (1942). Infections with the virus of Lymphocytic Choriomeningitis. *Medicine* 21, 65–94. doi: 10.1097/00005792-194202000-00001

Fischer, S. A. (2008). Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV. *Transplantation* 86, 1327–1339. doi: 10.1097/TP.0b013e31818b6548

Fischer, S. A., Graham, M. B., Kuehnert, M. J., Kotton, C. N., Srinivasan, A., Marty, F. M., et al. (2006). Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N. Engl. J. Med.* 354, 2235–2249. doi: 10.1056/NEJMoa053240

Folk, S., Steinbecker, S., Windmeyer, J., MacNeil, A., Campbell, S., and Rollin, P. E. (2011). Lymphocytic Choriomeningitis with severe manifestations, Missouri, USA. *Emerg. Infect. Dis.* 17, 1973–1974. doi: 10.3201/eid1710.110911

Gardner-Santana, L. C., Norris, D. E., Fornadel, C. M., Hinson, E. R., Klein, S. L., and Glass, G. E. (2009). Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*). *Mol. Ecol.* 13, 2766–2778 doi: 10.1111/j.1365-294X.2009.04232.x

Glass, G. E., Childs, J. E., Korch, G. W., and LeDuc, J. W. (1988). Association of intraspecific aggression and hantavirus infection in wild rats *(Rattus norvegicus). Epidemiol. Infect.* 101, 459–472. doi: 10.1017/S0950268800054418

Glass, G. E., Childs, J. E., Korch, G. W., and LeDuc, J. W. (1989). *Comparative Ecology and Social Interactions of Norway Rats Rattus norvegicus, in Baltimore, Maryland. Vol 130* Occasional papers of the Museum of Natural History. The University of Kansas, 1–33.

Glass, G. E., Klein, S. L., Norris, D. E., and Gardner, L. C. (2016). Multiple paternity in urban Norway rats: extended ranging for mates. *Vector-borne Zoon. Dis.* 16, 342–348. doi: 10.1089/vbz.2015.1816

Glass, G. E., Livingstone, W., Mills, J. N., Hlady, W. G., Fine, J. B., Biggler, W., et al. (1998). Black Creek Canal Virus infection in Sigmodon hispidus in southern Florida. *Am. J. Trop. Med. Hyg.* 59, 699–703. doi: 10.4269/ajtmh.1998.59.699

Glass, G. E., Watson, A. J., LeDuc, J. W., and Childs, J. E. (1994). Domestic cases of hemorrhagic fever with renal syndrome in the United States. *Nephron* 68, 48–51. doi: 10.1159/000188086

Gregg, M. B. (1975). Recent outbreaks of lymphocytic choriomeningitis in the United States of America. *Bull.World Health. Org.* 52, 549–554.

Hannah, M. F., Bajic, V. B., and Klein, S. L. (2008). Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. *Brain Behav. Immun.* 22, 503–516. doi: 10.1016/j.bbi.2007.10.005

Hinson, E. R., Shone, S. M., Zink, M. C., Glass, G. E., and Klein, S. L. (2004). Wounding: the primary mode of Seoul virus transmission among male Norway rats. *Am. J. Trop. Med. Hyg.* 70, 310–317 doi: 10.4269/ajtmh.2004.70.310

Hotchin, J. (1971). The contamination of laboratory animals with lymphocytic choriomeningitis virus. *Am. J. Pathol.* 64, 747–769.

Hotchin, J., Sikora, E., Kinch, W., Hinman, A., and Woodall, J. (1977). Lymphocytic Choriomeningitis in a hamster colony causes infection of hospital personnel. *Science* 185, 1173–1174. doi: 10.1126/science.185.4157.1173

Hutchinson, K. L., Rollin, P. E., and Peters, C. J. (1998). Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected *Sigmodon hispidus. Am. J. Trop. Med. Hyg.* 59, 58–65. doi: 10.4269/ajtmh.1998.59.58

Ike, F., Bourgade, F., Ohsawa, K., Sato, H., Morikawa, S., Saijo, M., et al. (2007). Lymphocytic Choriomeningitis infection undetected by dirty-bedding sentinel monitoring and revealed after embryo transfer of an inbred strain derived from wild mice. *Comp. Med.* 57, 272–281.

Ishii, S. (1942). Studies on Songo fever. Japan. *Army Med. J.* 335, 1755–1758.

Jameson, L. J., Taori, S. K., Atkinson, B., Levick, P., Featherstone, C. A., van der Burgt, G., et. al. (2013). Pet rats as a source of Hantavirus in England and Wales, 2013. *Euro Surveill.*18:20415. doi: 10.2807/ese.18.09.20415-en

Jamieson, D. J., Kourtis, A. P., Bell, M., and Rasmussen, S. A. (2006). Lymphocytic choriomeningitis virus: an emerging obstetric pathogen? *Am. J. Obstet.Gynecol.* 194, 1532–1536. doi: 10.1016/j.ajog.2005.11.040

Kang, S. S., and McGavern, D. B. (2008). Lymphocytic choriomeningitis infection of the central nervous system. *Front. Biosci.* 13, 4529–4543. doi: 10.2741/3021

Kawamata, J. Yamanouchi, T., Dohmae, K., Miyamoto, H., Takahaski, M., Yamanishi, K., et al. (1987). Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. *Lab. Anim. Sci.* 37, 431–436.

Keiner, C. (2005). Wartime rat control, rodent ecology and the rise and fall of chemical rodenticides. *Endeavour* 29, 119–125. doi: 10.1016/j.endeavour.2005.07.004

Kerins, J. L., Koske, S. E., Kazmierczak, J., Austin, C., Gowdy, K., Dibernardo, A., et al. (2018). Outbreak of Seoul virus among rats and rat owners – United States and Canada, 2017. *MMWR Morb. Mortal. Wkly. Rep.* 67, 131–134. doi: 10.15585/mmwr.mm6704a5

Khaiboullina, S. F., and St. Jeor, S. C. (2002). Hantavirus immunology. *Viral Immunol.* 15, 609–625 doi: 10.1089/088282402320914548

Kim, B., Vincent, H., Heikki, H., Darouny, P., Jean-Pierre, H., and Philippe, B. (2009). Rodent-borne zoonotic viruses in Southeast Asia. *J. Nat. Sci.* 43, 94–105.

Kitano, M. (1944a). Research on epidemic hemorrhagic fever. *Manchoukuo Med. J.* 40, 191–209.

Kitano, M. (1944b). Studies concerning epidemic hemorrhagic fever. *J. Jpn. Infect. Dis. Soc.* 18, 303–317.

Klein, S. L., Bird, B. H., and Glass, G. E. (2000). Sex differences in Seoul virus infection are not related to adult sex steroid concentrations in Norway rats. *J. Virol.* 74, 8213–8217. doi: 10.1128/JVI.74.17.8213-8217.2000

Klein, S. L., Bird, B. H., and Glass, G. E. (2001). Sex differences in immune responses and viral shedding following Seoul virus infection in Norway rats. *Am. J. Trop. Med. Hyg.* 65, 57–63. doi: 10.4269/ajtmh.2001.65.57

Klein, S. L., Cernetich, A., Hilmer, S., Hoffman, E. P., Scott, A. L., and Glass, G. E. (2004a). Differential expression of immunoregulatory genes in male and female Norway rats following infection with Seoul virus. *J. Med. Virol.* 74, 180–190. doi: 10.1002/jmv.20163

Klein, S. L., Marson, A. L., Scott, A. L., Ketner, G., and Glass, G. E. (2002). Neonatal sex steroids affect responses to Seoul virus infection in male but not female Norway rats. *Brain Behav. Immun.* 16, 736–746. doi: 10.1016/S0889-1591(02)00026-0

Klein, S. L., Zink, M. C., and Glass, G. E. (2004b). Seoul virus infection increases aggressive behaviour in male Norway rats. *Anim. Behav.* 67, 421–429 doi: 10.1016/j.anbehav.2003.03.022

Klempa, B. (2018). Reassortment events in the evolution of hantaviruses. *Virus Genes* 54, 638–646. doi: 10.1007/s11262-018-1590-z

Knust, B., MacNeil, A., Wong, S. J., Backenson, P. B., Gibbons, A., Rollin, P. E., et al. (2011). Exposure to Lymphocytic Choriomeningitis Virus, New York, USA. *Emerg. Infect. Dis.* 17, 1324–1325. doi: 10.3201/eid1707.101349

Knust, B., Ströher, U., Edison, L., Albariño, C. G., Lovejoy, J., Armeanu, E., et al. (2014). Lymphocytic Choriomeningitis Virus in employees and mice at multipremises feeder-rodent operation, United States, 2012. *Emerg. Infect. Dis.* 20, 240–247. doi: 10.3201/eid2002.130860

Korch, G. W., Childs, J. E., Glass, G. E., and LeDuc, J. W. (1989). Spatial and temporal analyses and host range of hantaviral infections within small mammal communities of Baltimore, Maryland USA. *Am. J. Trop. Med. Hyg.* 41, 230–240. doi: 10.4269/ajtmh.1989.41.230

Lambpropolous, S., Fine, J. B., Perbeck, A., Torres, D., Glass, G. E., McHugh, P., et al. (1999). Rodent control in urban areas: an interdisciplinary approach. *J. Environ. Health* 61, 12–17.

Lecompte, E., ter Meulen, J., Emonet, S., Daffis, S., and Charrel, R. N. (2007). Genetic identification of Kodoko virus, a novel arenavirus of the African pigmy mouse (*Mus nannomys* minutoides) in West Africa. *Virology* 364, 178–183. doi: 10.1016/j.virol.2007.02.008

LeDuc, J. W., Ksiazek, T. G., Rossi, C. A., and Dalrymple, J. M. (1990). A retrospective analysis of sera collected by the Hemorrhagic Fever Commission during the Korean conflict. *J. Infect. Dis.* 162, 1182–1184. doi: 10.1093/infdis/162.5.1182

LeDuc, J. W., Smith, G. A., Childs, J. E., Pinheiro, F. P., Maiztegui, J. I., Niklasson, B., et al. (1986). Global survey of antibody to Hantaan-related viruses among peridomestic rodents. *Bull. WHO* 64, 139–144.

Lee, H. W., Baek, L. J., and Johnson, K. M. (1982). Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic-fever, from wild urban rats. *J. Infect. Dis.* 146, 638–644. doi: 10.1093/infdis/146.5.638

Lee, H. W., and Johnson, K. M. (1982). Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic-fever. *J. Infect. Dis.* 146, 645–651. doi: 10.1093/infdis/146.5.645

Lee, H. W., Lee, P. W., and Johnson, K. M. (1978). Isolation of etiologic agent of Korean hemorrhagic-fever. *J. Infect. Dis.* 137, 298–308.

Lee, P. W., Yanagihara, R., Gibbs, C. J. Jr., and Gajdusek, D. C. (1986). Pathogenesis of experimental Hantaan virus infection in laboratory rats. *Arch. Virol.* 88, 57–66. doi: 10.1007/BF01310890

Lehmann-Grube, F. (1963). Lymphocytic choriomeningitis in the mouse. II. Establishment of carrier colonies. *Archiv. Virusforsch.* 14, 353–357.

Lehmann-Grube, F. (1971). "Lymphocytic Choriomeningitis virus,". in *Virology Monographs 10, Handbook of Virus Research*, eds S. Gard

Hallauer and C. K. F. Meyer (New York, NY: Springer-Verlag), 339–339. doi: 10.1007/978-3-7091-8276-5

Lehmann-Grube, F. (1984). Portrait of viruses: arenaviruses. *Intervirology* 22, 121–145. doi: 10.1159/000149543

Lewis, A. M., Rowe, W. P., Turner, H. C., and Huebner, R. J. (1965). Lymphocytic-Choriomeningitis virus in hamster tumor: spread to hamsters and humans. *Science* 150, 363–364. doi: 10.1126/science.150.3694.363

Li, W., and Klein, S. L. (2012). Seoul virus-infected rat lung endothelial cells and alveolar macrophages differ in their ability to support virus replication and induce regulatory T cell phenotypes. *J. Virol.* 86, 11845–11855 doi: 10.1128/JVI.01233-12

Lloyd, G., Bowen, E. T. W., Jones, N., and Pendry, A. (1984). HFRS outbreak associated with laboratory rats in UK. *Lancet* 1, 1175–1176. doi: 10.1016/S0140-6736(84)91413-2

Lloyd, G., and Jones, N. (1986) Infection of laboratory workers with hantavirus acquired from immunocytomas propagated in laboratory rats. *J. Infect.* 12, 117–125. doi: 10.1016/S0163-4453(86)93533-4

Long, J. L. (2003). *Introduced Mammals of the World*. Collingwood, VIC: CSIRO Publishing. doi: 10.1071/9780643090156

Lundkvist, A., Verner-Carlsson, J., Plyusnina, A., Forslund, L., Feinstein, R., and Plyusnin, A. (2013). Pet rat harbouring Seoul Hantavirus in Sweden, June 2013. *Euro Surveill.*18:20521. doi: 10.2807/1560-7917.ES2013.18.27.20521

MacNeil, A., Ströher, U., Farnon, E., Campbell, S., Cannon, D., Paddock, C. D., et al. (2012). Solid organ transplant–associated Lymphocytic Choriomeningitis, United States, 2011. *Emerg. Infect. Dis.* 18, 1256–1262. doi: 10.3201/eid1808.120212

Maris, C. H., Chappell, C. P., and Jacob, J. (2007). Interleukin-10 plays an early role in generating virus-specific T cell anergy. *BMC Immunol.* 8:8. doi: 10.1186/1471-2172-8-8

Mathur, G., Yadav, K., Ford, B., Schafer, I. J., Basavaraju, S. V., Knust, B., et al. (2017). High clinical suspicion of donor-derived disease leads to timely recognition and early intervention to treat solid organ transplant-transmitted lymphocytic choriomeningitis virus. *Transpl. Infect. Dis.* 19. doi: 10.1111/tid.12707

Mattar, S., Guzman, C., and Figueiredo, A. T. (2015). Diagnosis of hantavirus infection in humans Expert Rev. *Anti-Infect. Ther.* 13, 939–946. doi: 10.1586/14787210.2015.1047825

McAllister, R. C., and Jonsson, C. B. (2014). Hantaviruses: past, present and future. *Future Virol.* 9, 87–99. doi: 10.2217/fvl.13.113

McElhinney, L. M., Marston, D. A., Pounder, K. C., Goharriz, H., Wise, E. L., Verner-Carlsson, J., et al. (2017). High prevalence of Seoul hantavirus in a breeding colony of pet rats. *Epidemiol. Infect.*145, 3115–3124. doi: 10.1017/S0950268817001819

McKenna, P., van der Groen, G., Hoofd, G., Beelaert, G., Leirs, H., et al. (1992). Eradication of hantavirus infection among laboratory rats by application of caesarian section and a foster mother technique. *J. Infect.* 25, 181–188. doi: 10.1016/0163-4453(92)94035-V

Meritet, J. F., Krivine, A., Lewin, F., Poissonnier, M. H., Poizat, R., Loget, P., et al. (2009). A case of congenital lymphocytic choriomeningitis virus (LCMV) infection revealed by hydrops fetalis. *Prenat. Diagn.* 29, 626–627. doi: 10.1002/pd.2240

Meyer, H. M. Jr., Johnson, R. T., Crawford, I. P., Dascomb, H. E., and Rogers, N. G. (1960). Central nervous system syndromes of viral etiology. *Am. J. Med.* 29, 334–347. doi: 10.1016/0002-9343(60)90029-2

Montali, R. J., Ramsay, E. C., Stephensen, C. B., Worley, M., Davis, J. A., and Holmes, K. V. (1989). A new transmissible viral hepatitis of marmosets and tamarins. *J. Infect. Dis.* 160, 759–765. doi: 10.1093/infdis/160.5.759

Montali, R. J., Scanga, C. A., Pernikoff, D., Wessner, D. R., Ward, R., and Holmes, K. V. (1993). A common-source outbreak of callitrichid hepatitis in captive tamarins and marmosets. *J. Infect. Dis.* 167, 946–950. doi: 10.1093/infdis/167.4.946

Morita, C., Matsuura, Y., Fujji, H., Joh, K., Baba, K., Kato, M., et al. (1991). Isolation of lymphocytic choriomeningitis virus from wild house mice (*Mus musculus*) in Osaka Port, Japan. *J. Vet. Med. Sci.* 53, 889–892. doi: 10.1292/jvms.53.889

Moskophidis, D., Lechner, F., Pircher, H., and Zinkernagel, R. M. (1993). Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* 362, 758–761. doi: 10.1038/362758a0

Murphy, R. G., W. R., and Hide, G. (2005). "Population biology of the urban house mouse (*Mus musculus*) in the U.K.," in *Fifth International Conference of Urban Pests*, eds C. Y. Lee and R. W. (Singapore).

Nichol, S. T., Spiropoulou, C. F., Morzunov, S., Rollin, P. E., Ksiazek, T. G., Feldmann H., et al. (1993). Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 262, 914–917. doi: 10.1126/science.8235615

Nuzum, E. O., Rossi, C. A., Stephenson, E. H., and LeDuc, J. W. (1988). Aerosol transmission of Hantaan and related viruses to laboratory rats. *Am. J. Trop. Med. Hyg.* 38, 636–640. doi: 10.4269/ajtmh.1988.38.636

Palacios, G., Druce, J., Du, L., Tran, T., Birch, C., Briese, T., et al. (2008). A new arenavirus in a cluster of fatal transplant-associated diseases. *N. Eng. J. Med.* 358, 991–998. doi: 10.1056/NEJMoa073785

Penaloza-MacMaster, P., Kamphorst, A. O., Wieland, A., Araki, K., Iyer, S. S., West, E. E., et al. (2014). Interplay between regulatory T cells and PD-1 in modulating T cell exhaustion and viral control during chronic LCMV infection. *J. Exp. Med.* 211, 1905–1918. doi: 10.1084/jem.20132577

Peters, C. J. (2006). Lymphocytic Choriomeningitis virus — an old enemy up to new tricks. *N. Engl. J. Med.* 354, 2208–2211. doi: 10.1056/NEJMp068021

Petras, M. L. (1967). Studides of natural populations of Mus. I. Biochemical poly morphisms and their bearing on breeding structure. *Evolution* 21, 259–274.

Pocock, M. J. O., Hauffe, H. C., and Searle, J. B. (2005). Dispersal in house mice. *Biol. J. Linn. Soc.* 84, 565–583. doi: 10.1111/j.1095-8312.2005.00455.x

Reynes, J.-M., Carli, D., Bour, J.-B., Boudjeltia, S., Dewilde, A., Gerbier, G., et al. (2017). Seoul virus infection in humans, France, 2014-2016. *Emerg. Infect. Dis.* 23, 973–977. doi: 10.3201/eid2306.160927

Rivers, T. M., and Scott, T. F. M. (1935). Meinigitis in man caused by a filterable virus. *Science* 109, 439–440. doi: 10.1126/science.81.2105.439-a

Robertson, B. C., and Gemmell, N. J. (2004). Defining eradication units to control invasive pests. *J. Appl. Ecol.* 41, 1042–1048. doi: 10.1111/j.0021-8901.2004.00984.x

Robin, C., Perkins, E., Watkins, F., and Christley, R. (2017). Pets, purity and pollution: why conventional models of disease transmission do not work for pet rat owners. *Int. J. Environ. Res. Public Health* 14:E1526. doi: 10.3390/ijerph14121526

Rosen, A., and Ison, M. G. (2017). Screening of living organ donors for endemic infections: understanding the challenges and benefits of enhanced screening. *Transpl. Infect. Dis.* 19:e12633. doi: 10.1111/tid.12633

Sabattini, M. S., Barrera Oro, J. G., Maiztegui, J. I., Fernandez, D., Contigiani, M. S., and Diaz, G. E. (1970). Aislamiento de un Arenavirus relacionado con el de la coriomeningitis linfocitica (LCM) a partir de un *Mus musculus* capturado en zona endemica de fiebre hemorragica Argentina (FHA). *Rev. Soc. Argen. Microbiol.* 11, 182–184.

Sabattini, M. S., Barrera Oro, J. G., Maiztegui, J. I., and Ferradas, B. R. (1974). Actividad del virus de la Coriomeningitis linfocitica en el area endemica de Fiebre Hemorragica Argentina. II. Aislamiento a partir de un *Mus musculus* campestre capturado en el sudeste de Cordoba. *Medicina* 34, 313–313.

Salvato, M., P., Borrow, P., Shimomaye, E., and Oldstone, M. B. (1991). Molecular basis of viral persistence: a single amino acid change in the glycoprotein of lymphocytic choriomeningitis virus is associated with suppression of the antiviral cytotoxic T-lymphocyte response and establishment of persistence. *J. Virol.* 65, 1863–1869.

Salvato, M., Shimomaye, E., Southern, P., and Oldstone, M. B. (1988). Virus-lymphocyte interactions. IV. Molecular characterization of LCMV Armstrong (CTL+) small genomic segment and that of its variant, Clone 13 (CTL-). *Virology* 164, 517–522. doi: 10.1016/0042-6822(88)90566-1

Schmaljohn, C. S., Chu, Y. K., Schmaljohn, A. L., and Dalrymple, J. M. (1990). Antigenic subunits of Hantaan virus expressed by baculovirus and vaccinia virus recombinants. *J. Virol.* 64, 3162–3170

Schountz, T., Prescott, J., Cogswell, A. C., Oko, L., Mirowsky-Garcia, K., and Hjelle, A. P. (2007). Regulatory T cell-like responses in deer mice persistently infected with Sin Nombre virus. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15496–15501. doi: 10.1073/pnas.0707454104

Singleton, G. (1983). The social and genetic structure of a natural colony of house mice, mus musculus, at healesville wildlifesanctuary. *Austral. J. Zool.* 31, 155–166. doi: 10.1071/ZO9830155

Skinner, H. H., and Knight, E. H. (1973). Natural routes for post-natal transmission of murine lymphocytic choriomeningitis. *Lab. Anim.* 7, 171–184. doi: 10.1258/002367773781008597

Skinner, H. H., Knight, E. H., and Buckley, L. S. (1976). The hamster as a secondary reservoir host of lymphocytic choriomeningitis virus. *J. Hyg. Camb.* 76, 299–306. doi: 10.1017/S0022172400055194

Skinner, H. H., Knight, E. H., and Grove, R. (1977). Murine lymphocytic choriomeningitis: the history of a natural cross-infection from wild to laboratory mice. *Lab. Anim.* 11, 219–222. doi: 10.1258/002367777780936422

Smadel, J. E. (1953). Epidemic hemorrhagic fever. *Am. J. Public Health* 43, 1327–1330. doi: 10.2105/AJPH.43.10.1327

Smorodintsev, A. A., Al'tshuler, I. S., Dunaevskii, M. L., Kokhredze, K. A., Neustroev, V. D., and Churilov, A. V. (1944). Etiology and clinical observations of hemorrhagic nephroso-nephritis. *Medgiz Moscow* 26–47.

Smorodintsev, A. A., Chudakov, V. G., and Churilov, A. V. (1959). *Haemorrhagic Nephroso-Nephritis.* London: Pergamon Press.

Steele, J. H., Fernandez, P. J., and Baer, G. M. (1991). *The Natural History of Rabies*. Boca Raton, FL: CRC Press.

Stephensen, C. B. (1992). Prevalence of serum antibodies against Lymphocytic Choriomeningitis virus in selected populations from 2 United States cities. *J. Med. Virol.* 38, 27–31. doi: 10.1002/jmv.1890380107

Stephensen, C. B., Jacob, J. R., Montali, R. J., Holmes, K. V., Muchmore, E., Compans, R. W., et al. (1991). Isolation of an arenavirus from a marmoset with callitrichid hepatitis and its serologic association with disease. *J. Virol.* 65, 3995–4000.

Sukthana, Y. (2006). Toxoplasmosis: beyond animals to humans. *Trends Parasitol.* 22, 137–142. doi: 10.1016/j.pt.2006.01.007

Takimoto, K., Taharaguchi, M., Morikawa, S., Ike, F., Yamada, Y. K.,(2008). Detection of the antibody to Lymphocytic Choriomeningitis virus in sera of laboratory rodents infected with viruses of laboratory and newly isolated strains by ELISA using purified recombinant nucleoprotein. *Exp. Anim.* 57, 357–365. doi: 10.1538/expanim.57.357

Traub, E. (1936a). An epidemic in a mouse colony due to the virus of acute lymphocytic choriomeningitis. *J. Exp. Med.* 63, 533–547. doi: 10.1084/jem.63.4.533

Traub, E. (1936b). The epidemiology of Lymphocytic Choriomeningitis in white mice. *J. Exp. Med.* 64, 183–200. doi: 10.1084/jem.64.2.183

Traub, E. (1936c). Persistence of Lymphocytic Choriomeningitis virus in immune animals and its relation to immunity. *J. Exp. Med.* 63, 847–861. doi: 10.1084/jem.63.6.847

Traub, E. (1938). Factors influencing the persistence of choriomenigitis virus in the blood of mice after clinical recovery. *J. Exp. Med.* 68, 229–250. doi: 10.1084/jem.68.2.229

Traub, E. (1939). Epidemiology of lymphocytic choriomeningitis in a mouse stock observed for four years. *J. Exp. Med.* 69, 801–817. doi: 10.1084/jem.69.6.801

Traub, R., and Wisseman, C. L. Jr. (1978). Korean hemorrhagic fever. *J. Infect. Dis.* 138, 267–272. doi: 10.1093/infdis/138.2.267

Umenai, T., Lee, P. W., Toyoda, T., Yoshinaga, K., Horiuchi, T., Lee, H. W., et al. (1979). Korean haemorrhagic fever in staff in an animal laboratory. *Lancet* 313, 1314–1316. doi: 10.1016/S0140-6736(79)91948-2

van den Pol, A. N. (2006). Viral infections in the developing and mature brain. *Trends Neurosci.* 29, 398–406. doi: 10.1016/j.tins.2006.06.002

Vanzee, B. E., Douglas, R. G. Jr., Betts, R. F., Bauman, A. W., Fraser, D. W., and Hinman, A. R. (1975). Lymphocytic choriomeningitis in University Hospital personnel. *Am. J. Med.* 58, 803–809. doi: 10.1016/0002-9343(75)90635-X

Wahl-Jensen, V., Chapman, J., Asher, L., Fisher, R., Zimmerman, M., et al. (2007). Temporal analysis of Andes virus and Sin Nombre virus infections of Syrian hamsters. *J. Virol.* 81, 7449–7462. doi: 10.1128/JVI.00238-07

Williams, S. H., Che, X., Garcia, J. A., Klena, J. D., Lee, B., Muller, D., et al. (2018). Viral diversity of house mice in New York City. *mBio* 9:01354-17. doi: 10.1128/mBio.01354-17

Wilson, D. E., and Reeder, D. M. (2005). *Mammal Species of the World, 2nd Edn.* Baltimore, MD: Johns Hopkins University Press.

Wooley, J. G., Stimpert, F. D., Kessel, J. F., and Armstrong, C. (1939). A Study of human sera antibodies capable of neutralizing the virus of Lymphocytic Choriomeningitis. *Public Health Rep.* 54, 938–944. doi: 10.2307/4582902

Yanagihara, R., Amyx, H. L., and Gajdusek, D. C. (1985). Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (*Clethrionomys glareolus*). *J. Virol.* 55, 34–38.

Zhang, X.-K., Takashima, I., and Hashimoto, N. (1988). Role of maternal antibody in protection from hemorrhagic fever with renal syndrome virus infection in rats. *Arch. Virol.* 103, 253–265. doi: 10.1007/BF01311097

Zhou, X., Ramachandran, S., Mann, M., and Popkin, D. (2012). Role of Lymphocytic Choriomeningitis Virus (LCMV) in understanding viral immunology: past, present and future. *Viruses* 4:2650. doi: 10.3390/v4112650

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Bartonella Bacteria in Urban Rats: A Movement From the Jungles of Southeast Asia to Metropoles Around the Globe

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Despite the widespread recognition of the risks of disease transmission associated with international trade in domestic animals and movement of exotic animals, less is known about the role of rats in carrying pathogens between continents. The genus *Bartonella*, a highly prevalent and extremely diverse group of bacteria, includes species that are excellent sentinel organisms for evaluating the transoceanic and intra-continental movement of the pathogens carried by rats of the genus *Rattus*. The patterns of spatial distribution, occurrence, and genetic diversity of *Bartonella* species infecting rats and their arthropod ectoparasites depend on the geographic locations within metropolitan areas of the Americas, Africa, Asia, and Europe. One of the points addressed in this review is a comparison of the diversity of *Bartonella* species carried by rats in their original habitats in Southeast Asia and in the cities occupied by rats recently. The invasion of *Rattus* rats into new urban territories create significant risk for human health.

Keywords: bacteria, *Bartonella*, invasive species, movement ecology, rats, *Rattus,* urban zoonoses

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INTRODUCTION

Commensal Rats and Infectious Agents Carried by Rats in Urban Areas

People create dramatically new environments. This is especially evident in urban settings, which may favor some mammalian species that might become "synanthropic." A number of factors contribute to the public health threat presented by synanthropic animals. According to the calculations provided by McFarlane et al. (2012), human cases of zoonotic infectious diseases caused by synanthropic animals happen 15 times more frequently compared to wild animals. Urban territories may provide suitable conditions for reproduction of arthropods serving as vectors for vector-borne diseases because of some specific environmental changes, such as attenuation of the temperature range and humidity (Shochat et al., 2006; Bradley and Altizer, 2007). The environments in cities have higher land surface temperature compared to surrounding natural habitats, so-called "urban heat islands" (Grimmond, 2007). The urban heat islands represent an example of the numerous potential environmental changes caused by urbanization that can affect distribution and prevalence of zoonotic and vector-borne diseases in cities and suburban areas.

Urban development changes rodent communities dramatically. Among mammalian species adapted to life in cities, certain species of the genus *Rattus* play especially evident and important roles because of their close association with human activities (Battersby et al., 2008). Living in close proximity to human houses and having high exploratory activity, rats frequently encounter people

in cities and small towns. An extensive questionnaire conducted among residents of Baltimore, Maryland ($N = 1{,}363$) showed 64% of respondents seen rats on streets and alleys, 6% of residents noticed rats inside houses, and 1.2% of residents reported bites by rats (Childs et al., 1991). Microclimatic conditions in cities can affect development of the fleas found on rats (Krasnov et al., 2001) and, therefore, influence the transmission of some vector-borne bacteria. In addition, the urban environment affect availability of resources, specifically food, for rodents (Cevidanes et al., 2017), promoting higher concentration, and density of rats that in turn can facilitate density-dependent transmission of various zoonotic pathogens carried by these animals.

Rattus Rats as Invasive Species

Over 60 species belong to the genus *Rattus* (Musser and Carlton, 2005). Of these, only three species, the Norway rat (*R. norvegicus*), the black (roof) rat (*R. rattus*), and the Oriental house rat (*R. tanezumi*), have dispersed around the globe and colonized urban settings in most countries, and the range of the fourth invasive species, the Pacific rat (*R. exulans*), is limited to tropical Asia-Pacific areas (Kosoy et al., 2015). Rats of the genus *Rattus* became close neighbors to humans at a very early stage of human civilization. However, during the last century, distribution of rats has dramatically expanded to new geographic regions, and the rat invasion of the cities in different countries led to dramatic challenges (Khlyap et al., 2012). Importantly, the process of rat invasion from the region of their origin to other places continues. In the past, the distribution of rats depended mainly on cart traffic and ship routes, the latter being the leading means of crossing the oceans. In the modern world, the role of railroad, truck, and airplane in transporting rats is growing (Khlyap et al., 2016).

Application of molecular tools for barcoding animal species has challenged taxonomic identification of rats. Analysis of mitochondrial DNA of rats, previously identified as *R. rattus*, demonstrated the complex of separate species within the *R. rattus*-complex (Aplin et al., 2011). A recent survey of rats belonging to this complex collected across their global range and conducted by Aplin et al. (2011) allowed discriminating several genetic lineages within the black rat complex. Their investigations demonstrated that a diversification of these lineages happened in the early Middle Pleistocene within South Asia, southern and northern Indochina, and in the region close to Himalayas. These authors also identified two other currently recognized *Rattus* species as potential derivatives of a paraphyletic *R. rattus* (Aplin et al., 2011). Interestingly, their results demonstrated that three of four phylogenetic lineages within the *R. rattus* complex happened in prehistoric times. The distribution of particular genotypes matches historically documented patterns of human dispersal and trade. Based on this analysis, Aplin et al. (2011) concluded that commensalism arose multiple times in black rats and in widely separated geographic regions. Importantly, such a regionalism may account for spreading of pathogens associated with *R. rattus* (Aplin et al., 2011). Examining multiple samples of this species from different parts of the world, Aplin et al. (2011) reported ecologically and morphologically similar lineages of rats with distinct histories of invasion to other geographic regions. One of the lineages, designated as *R. rattus* I, has dispersed around the globe and exists alongside humans, while rats of another lineage (designated as *R. rattus* IV) have not spread beyond Bornean Malaysia, Indonesia, and the Philippines (Aplin et al., 2011; Lack et al., 2012).

Ecology of Rats in Urban Settings

Rodents in urban environments often show specific adaptations (Khlyap et al., 2012, 2016). Sometimes it may be challenging to define urban areas using ecological perspectives, especially when suburban areas are considered. It is also important to remember that ecological conditions in urban areas vary among countries. Specifically, the distribution of rats may greatly contrast between countries in the South and Southeast Asia where *Rattus* rats occupy practically all habitats and Europe and North America where rats are commonly restricted to city limits (Khlyap et al., 2012). Himsworth et al. (2014a) demonstrated that population density of urban rats varied significantly over short distances. Populations of rats are often largest in high-density residential areas (Khlyap et al., 2016).

Some big cities are particularly favorable places for urban rats because of their aging infrastructure, high moisture, and poverty rates. Easterbrook et al. (2005) estimated an outdoor Norway rat population in residential neighborhoods of Baltimore at around 48,000 individuals. Though these rats can disperse over long distances, investigations of marked animals demonstrated that they tend to live within small individual territories, sometimes not extending beyond a single building. Genetic analysis using microsatellite markers showed that rats demonstrate strong site fidelity (Gardner-Santana et al., 2009). However, there was evidence of infrequent, long-distance movements within the city indicated by capturing some rats 2–11.5 km away from the locations assigned based on the genetic analysis (Gardner-Santana et al., 2009). Among factors influencing rat presence and abundance, Himsworth et al. (2014b) suggested building condition and specific land use. In industrialized countries, rats commonly occupy sewer system of cities (Lund, 2015).

Pathogens and Movement Ecology of Animals

As invasive animal species rapidly become more prevalent in many parts of the world, relations between previously disconnected animal populations can promote the spread of pathogens carried by these animals (Crowl et al., 2008). On the other side of the coin, infectious agents detected in invasive animals may illustrate the "tracks" left during the spreading of their mammalian hosts. Genetic studies of animals provide irreplaceable tools for deciphering routes of invasion, but characterization of accompanying micro- and macro-parasites can provide additional support for such a goal. An assessment of risk of infectious diseases introduced by invasive animal hosts highlights importance of such information. Nevertheless, the selection of specific microbial species that can serve as markers for measuring the movement of animal hosts is not a trivial task. Clearly, the selected microorganisms should be prevalent in animal populations, but not too much so otherwise

the omnipresent infections cannot be good indicators of animal movement. Secondly, these microbial species should be highly specific to their animal hosts to reflect the long history of coevolution between animals and microbes. Finally, their genetic variability should be high enough to reflect the routes of dispersion of the animals hosting these microbial agents.

Bartonella Species as Sentinel Organisms

Bartonella species comprise Gram-negative bacteria parasitizing mammalian erythrocytes and endothelial cells (Birtles, 2005). Infecting a wide variety of hosts, *Bartonella* may present in these animals as a subclinical and persistent bacteremia (Schülein et al., 2001). *Bartonella* species are an extremely diverse group of bacteria infecting various mammalian species, especially rodents (Kosoy et al., 2018), that also spans the symbiont-pathogen continuum (Segers et al., 2017). Moreover, these "vertebrate host-arthropod vector-bartonellae" tripartite systems appear to be globally distributed, phylogenetically complex, and provide a popular tool for ecological comparative analyses (Buffet et al., 2013; Brook et al., 2017; Kosoy et al., 2018). The rodent habitat represents an important factor for the transmission cycle of *Bartonella* in nature (Gutiérrez et al., 2015). Analyses of genetic diversity of *Bartonella* species based on sequencing approaches can be informative for comparing bacterial prevalence and diversity in rat populations across various spatial and temporal scales. However, the effect of urbanization on *Bartonella* prevalence and diversity in rat populations has never been sufficiently analyzed. The objectives of ecological studies determine the level of discrimination between compared strains or genotypes. In most situations, the investigators report discrimination of bacteria at the species level or compare sequence identity with a specific *Bartonella* type strain (Kosoy et al., 2018). In the absence of sequence data, reporting PCR-positive samples alone may overestimate bartonella prevalence in such ecological studies. Therefore, Kosoy et al. (2018) advocated that studies of prevalence of *Bartonella* should adhere to the standard of reporting only sequence-positive samples.

METHODS

We thoroughly analyzed published literature concerning identification of *Bartonella* species in rats. For this review, we examine prevalence and diversity of *Bartonella* in rats belonging to the genus *Rattus*. Only in few instances, strictly for comparative purposes, we provided data on other mammalian species co-habiting with rats of *Rattus*. We conducted a literature search by various search engines, including PubMed, Scopus, BioOne, Medline, ScienceResearch, Google Scholar, OVID Medicine, and Web of Science. In the search, we used the following keywords: "*Bartonella* AND Rats," "*Bartonella* AND Rodents," "Bacteria AND Rattus," "Rat-Borne Diseases," "Rodent-Borne-Diseases," "Urban Bacterial Diseases," "Urban Rodents AND Pathogens," and their combinations. We analyzed data from serological, molecular, and bacteriological detection of *Bartonella* in rats. Analyzing data obtained from different assays, we have given a priority to the results that included an identification of *Bartonella* species and genotypes.

RESULTS AND DISCUSSION

Rattus Rat-Adapted *Bartonella* Species

Overall, *Bartonella* species and genotypes found in *Rattus* rats are highly specific for rodents belonging to this genus (Kosoy et al., 2012; Buffet et al., 2013). Many rat-adapted *Bartonella* species have a worldwide distribution (Buffet et al., 2013; Hayman et al., 2013). The most prevalent *Bartonella* species, such as *B. elizabethae, B. tribocorum, B. rattimassiliensis,* and *B. queenslandensis*, are phylogenetically clustered in a well-demarcated lineage (**Figure 1**). Originally, all these species were described based on the sequence distances between the species for several housekeeping gene markers (Heller et al., 1998; Gundi et al., 2004, 2009). However, the question remains how to recognize the status of strains that occupy intermediate positions between the described *Bartonella* species. This question was not unique for *Bartonella* strains found in rats. To solve this problem, Kosoy et al. (2012) proposed to use a combination of genetic markers and ecological parameters for delineation of species complexes that include closely related genospecies, named strains, and unique genotypes carried by ecologically similar mammalian hosts. Following this proposal, the *B. elizabethae* complex *sensu lato* represents a large group of species and strains associated with the Old World rats (Kosoy et al., 2012; Buffet et al., 2013). A well-characterized, but unnamed strain "Tel-Aviv" also belongs to the *B.elizabethae* species complex (Harrus et al., 2009).

In addition, three more *Bartonella* species (*B. phoceensis, B. coopersplainsensis,* and *B. rochalimae*) infect *Rattus* rats worldwide (Buffet et al., 2013). Of these three species the first two (*B. phoceensis* and *B. coopersplainsensis*) are typical for *Rattus* rats, while *B. rochalimae* is an ubiquitous bacterial species detected in a wide range of mammals, and is especially common in wild carnivores and their fleas (Bai et al., 2016). *B. phoceensis* is a bacterium originally isolated from the blood of the rats of *R. norvegicus* from the city of Marseille, France (Gundi et al., 2004). *Bartonella coopersplainsensis* was isolated from the blood of a Cape York rat (*Rattus leucopus*) in Australia (Gundi et al., 2009).

Although stressing the high level of host-specificity for *Rattus* among *B. elizabethae*-like species, we have to admit that these species can also infect other mammals, e.g., *Bandicota* rats and Asian house shrews (*Suncus murinus)* from Bangladesh and Nepal (Bai et al., 2007; Gundi et al., 2010); Brush-furred rats (*Lophuromys* sp.) from Tanzania (Gundi et al., 2012); Namaqua rock rats (*Aethomys namaquensis*) and Bushveld gerbils (*Tatera leucogaster*) from South Africa (Pretorius et al., 2004); and Cairo spiny mice (*Acomys cahirinus*) from Israel (Morick et al., 2009). The unexpectedly broad host range of *B. elizabethae*-like species might be explained by the commonality of fleas that infest various rodent species. Thus, *B. elizabethae, B. tribocorum,* and *B. queenslandensis* DNA have been detected in *Xenopsylla* fleas collected not only from *Rattus* rats, but also from gerbils, *Mus*

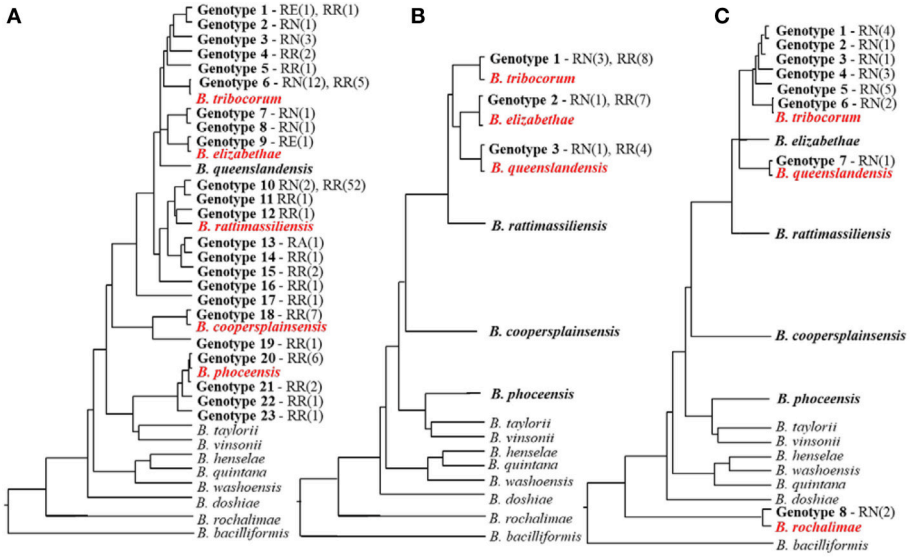

FIGURE 1 | Diversity of *Bartonella* species and genotypes found in *Rattus* rats from rural Thailand **(A)**, an urban slum in Nairobi, Kenya **(B)**, and downtown Los-Angeles, California, U.S. **(C)** and their schematic phylogenetic relations. The names of *Bartonella* species found in a particular study are in red. The numbers of detected genotypes do not correspond to each other and are given solely for comparative purpose. The phylogenetic trees represent phylogenetic relationship of the *gltA* sequences of the named *Bartonella* species and unnamed genotypes obtained from three separate studies (Bai et al., 2009; Gundi et al., 2012; and Halliday et al., 2015) following the same methodology at one laboratory (CDC, Fort Collins, Colorado). The names of rat species are abbreviated as RN for *R. norvegicus* and RR for *R. rattus*; the number of isolates obtained from each species is in parentheses.

mice, and shrews worldwide (Tsai et al., 2010; Billeter et al., 2011; Bitam et al., 2012).

BARTONELLA INFECTIONS IN URBAN POPULATIONS OF RATS

Asia

Bangladesh

Prevalence of *Bartonella* bacteria in *R. rattus* collected in Kamalapur, a low socioeconomic residential area of Dhaka, was 32.3% (32/99) (Bai et al., 2007). This rate was lower than that observed in two other co-habiting mammalian species: lesser bandicoot rats *Bandicota bengalensis* (63.2%) and house shrews *Suncus murinus* (42.9%). Bacteriological observations of small mammals captured in Dhaka indicated a diverse assemblage of genetic variants of *Bartonella* (Bai et al., 2007). The isolates obtained from *R. rattus* belonged to three groups, none of which aligned closely with previously described *Bartonella* species, whereas isolates obtained from lesser bandicoot rats *Bandicota bengalensis* were much closer or identical to *B. elizabethae* or *B. tribocorum*. Importantly, bartonellae isolated from two black rats captured in Dhaka were identical by the *gltA* gene to the isolates previously found in rats from Porto Santo Island, Portugal and New Orleans, Louisiana, USA. Later, multiple isolates similar to this type were found in *R. rattus* rats from Tel Aviv, Israel (Harrus et al., 2009).

China

The first study, which demonstrated a high prevalence of *Bartonella* in *Rattus* rats in Asian cities was conducted in Yunnan Province, the southwestern part of the mainland China (Ying et al., 2002). Culturing blood of rats from three cities (Yiliang, Baoshan, and Jianchuan) revealed 41.4% (24/58) *Bartonella*-positive animals among *R. tanezumi* subsp. *flavipectus* and 42.9% (3/7) among *R. norvegicus*. Rats in every investigated city along the coast of Fujian Province were infected by *Bartonella* species: 9.3% in Ningde, 9.5% in Fuzhou, 9.4% in Putian, 28.2% in Quanzhou, 17.4% in Xiamen, and 13.3% in Zhangzhou (Ye et al., 2009). Despite considerable heterogeneity and varying degrees of relatedness to *B. elizabethae*, all isolates from urban rats belonged to the same phylogenetic lineage. Isolates from rats from Fujian coastal regions belonged to three species: *B. elizabethae, B. queenslandensis,* and *B. tribocorum* (Ye et al., 2009).

Indonesia

Microscopic examination of blood smears of rodents from the Greater Jakarta area revealed 6.0% (13/218) *Bartonella*-positive rodents. Of 79 *R. tanezumi*, 49 *R. norvegicus,* and one *R. exulans* captured in three study sites (Bambu Apus, Penjaringan Harbor, and Ragunan Zoo), seven *R. tanezumi* rats and one *R. norvegicus* rat were positive for *Bartonella* species (Winoto et al., 2005). Three *Bartonella* species (*B. phoceensis, B. rattimassiliensis,* and *B. elizabethae*) were found in rats (*R. tanezumi* and *R. norvegicus*) from Jakarta (Winoto et al., 2005).

Japan

Interesting results were reported after investigation of rats collected in two cities and four suburban areas of Japan (Inoue et al., 2008). *Bartonella* isolates were obtained from *R. rattus* from suburban areas in Nakanoshima Island (2/4), Yoroshima Island (10/17), Tokunoshima Island (4/12), and Main Island (2/6). At

the same time, all *R. rattus* rats captured in cities of Yokohama ($n = 255$) and Shimoda ($n = 3$) were free of *Bartonella*. All *R. norvegicus* from the cities ($n = 85$) and suburban areas ($n = 20$) were negative for *Bartonella* (Inoue et al., 2008). Investigating *R. rattus* rats captured from several places in Japan, Inoue et al. (2008) identified *B. tribocorum*, *B. elizabethae, B. phoceensis,* and *B. rattimassiliensis* based on two genetic markers.

Lao P.D.R

A molecular survey of rats was conducted in three urban areas of Laos (Angelakis et al., 2009). *Bartonella* DNA was found in 10.1% of *R. rattus* ($n = 79$) and 30.4% of *R. exulans* ($n = 23$) from Vientiane City, the largest city of Laos, which is located near its border with Thailand. Nine percent of *R. rattus* ($n = 141$) from the city of Luang Prabang in northern part of Laos were positive, as were 20.1% of *R. rattus* ($n = 159$) from a town in Luang Namtha Province near the border with Yunnan, China (Angelakis et al., 2009). Angelakis et al. (2009) identified three well-characterized *Bartonella* species (*B. phoceensis, B. elizabethae,* and *B. tribocorum*) and two additional genotypes (Lao/Nh1 and Lao/Nh2) related to *B. tribocorum*. In *R. rattus* rats, the authors found *B. phoceensis* ($n = 1$), *B. elizabethae* ($n = 2$), *B. tribocorum* ($n = 2$), and Lao/Nh2 ($n = 3$); while in *R. exulans* rats, the predominant species was *B. tribocorum* ($n = 5$) with single positive rats infected with either *B. elizabethae* or *B. phoceensis* ($n = 1$) (Angelakis et al., 2009).

Malaysia

Urban rats were captured as part of a pest management program in Kuala Lumpur ($n = 59$) and Pulau Pinang ($n = 36$). Of the 95 bacteriologically and molecularly tested rats, 13.5% of *R. rattus* ($n = 58$) and 13.8% of *R. norvegicus* ($n = 37$) were positive for *Bartonella* (Tay et al., 2014). Five species of *Bartonella* (*B. tribocorum, B. rattimassiliensis, B. coopersplainsensis, B. elizabethae*, and *B. queenslandensis*) were identified (Tay et al., 2014).

Nepal

Bartonella was detected in 39 (43.3%) of 90 *R. rattus* subsp. *brunneusculus* captured in three cities: Bhaktapur (33.3%; 12/26), Kathmandu (40.6%; 13/32), and Lalitpur (43.8%; 14/32) (Gundi et al., 2010). There were five species of *Bartonella* in *R. rattus* subsp. *brunneusculus* collected in three urban areas in Nepal, including *B. elizabethae* ($n = 5$), *B. coopersplainsensis* ($n = 3$), *B. tribocorum* (4), *B. queenslandensis* ($n = 2$), and *B. phoceensis* ($n = 1$). In addition, three genotypes were different from any described *Bartonella* species (Gundi et al., 2010).

Singapore

A molecular survey of commensal rodents in Singapore resulted in detection of *Bartonella* DNA in 75% (3/4) of *R. norvegicus* and 34.5% (10/29) of *R. tanezumi* (Neves et al., 2018). Annandale's rats (*R. annandalei*) captured outside the urban areas were negative for *Bartonella*. The only *R. exulans* captured in a city park was also free of *Bartonella*. Characterization of *Bartonella* genotypes circulating in rat populations within Singapore has revealed five species (Neves et al., 2018). Interestingly, the composition of *Bartonella* species differed depending on the rat species. *B. coopersplainsensis, B. elizabethae*, *B. grahamii,* and *B. phoceensis* were found in *R. tanezumi*; whereas *B. tribocorum, B. rattimassiliensis, B. grahamii,* and *B. queenslandensis* were found in *R. norvegicus*. A smaller number of *Bartonella* species was in *R. exulans*: *B. tribocorum, B. rattimassiliensis,* and *B. phoceensis* (Neves et al., 2018).

Taiwan

There were several studies of urban rats in Taiwan. The investigation of rodents captured in the Taichung area, including markets in the urban area, has found 52.7% of *R. norvegicus* ($n = 169$), 10% of *R. rattus* ($n = 10$), and 66.7% of *R. losea* ($n = 3$) (Hsieh et al., 2010) were positive for *Bartonella*. Among the 182 rats tested from Taichung, the city located in central Taiwan, the cultured organisms belonged to five species of *Bartonella* (Hsieh et al., 2010). Strains closely related to *B. tribocorum* and *B. elizabethae* were the most prevalent of them. In addition, *B. rattimassilensis*, *B. grahamii*, and *B. phoceensis* were isolated from *R. norvegicus*. Several *R. norvegicus* rats were co-infected with different *Bartonella* species: 10 rats with *B. elizabethae*-like and *B. tribocorum*-like organisms and one rat with *B. phoceensis*-like and *B. tribocorum*-like organisms (Hsieh et al., 2010).

Another study in Taichung resulted in detection of *Bartonella* in 9.4% (5/53) of *R. norvegicus* and 33.3% (1/3) of *R. rattus* (Lin et al., 2008). In another study in Taiwan, the same species of *Bartonella* (*B. phoceensis, B. tribocorum, B. elizabethae*, and *B. rattimassiliensis*) were detected in rats, but, in addition, *B. queenslandensis* was also reported (Tsai et al., 2010). The *Bartonella* detected in two *R. norvegicus* rats captured on the university campus was *B. tribocorum*. As we will show later, the diversity of *Bartonella* species in rats from natural habitats can be much higher. Interestingly, Lin et al. (2008) reported isolation of strains closely related to *B. rochalimae* from *R. norvegicus* captured Taichung, Taiwan.

Thailand

In spite of many investigations of *Bartonella* infections in rats from the fields and forest in different parts of Thailand, there were very limited efforts to investigate urban rats. There is only one report of investigation of urban rats captured in Bangkok in 2008: *Bartonella* bacteria were cultured from *R. exulans* (55.6%; $n = 9$) and *R. rattus* (67.6%; $n = 34$), and none from three *R. norvegicus* (Kim et al., 2016).

Middle East

Cyprus

Of 622 rats (402 *R. norvegicus* and 220 *R. rattus* subsp. *frugivorus*) collected in 51 different sites in Cyprus, 10.5% were found seropositive for *Bartonella* antibodies (Psaroulaki et al., 2010). The authors of this study used *B. henselae* antigens and, more likely, the reported antibodies were cross-reactive with other *Bartonella* species. The authors, however, claimed that the seropositivity rate significantly correlated with the presence of cat fleas, but not with the presence of rat fleas.

Israel

Bartonella DNA was detected in spleen samples of 19 out of 79 (24%) *R. rattus* captured in 19 suburban sites ranged from the kibbutz Dafna in the north of Israel to the City of Beersheba in the south (Morick et al., 2009). *Bartonella* strains obtained from *R. rattus* rats captured in Tel-Aviv, Israel were characterized by sequencing multiple genetic loci (*gltA, ribC, rpoB, 16S RNA, groEL,* and the intergenic spacer region ITS) (Harrus et al., 2009). These strains were identical among themselves, and sequences of each of the analyzed genes indicated a closeness to one of the two *Bartonella* species (*B. tribocorum* and *B. elizabethae*), while being different from both of these species (Harrus et al., 2009). The characterization of these strains supports a hypothesis that it could be a separate species of *Bartonella.*

Turkey

In a study conducted in an urban area of Zongulda in the western Black Sea Region of Turkey, only one rat out of 14 investigated, including eight *R. rattus* and six *R. norvegicus* was found to be *Bartonella*-positive (Çelebi et al., 2015). A *Bartonella* isolate obtained from *R. rattus* rats captured in Zongulda was identified as *B. coopersplainsensis* (Çelebi et al., 2015), the species which has been previously reported in Australia and Southeastern Asia (Gundi et al., 2009; Jiyipong et al., 2012).

Africa

Benin

A molecular survey of *Bartonella* species in rats captured inside human residences and peridomestic areas was conducted in three zones of Cotonou where half of the population of the Republic of Benin resides (Martin-Alonso et al., 2016). *Bartonella* DNA was detected in 26.3% (5/19) of *R. norvegicus* captured in the zone 1 (20.8%), whereas none of the 110 *R. rattus* captured in the three zones were found positive for *Bartonella*. Three *Bartonella* species (*B. elizabethae, B. tribocorum,* and *B. rochalimae*) were found in spleens of *R. norvegicus* rats from an urban area near Nokoue Lake in Benin (Martin-Alonso et al., 2016).

DR Congo

In studies conducted in Djalusene, Kpandruma, Rethy, and Zaa, *R. rattus* was the only rat species captured inside houses and the domestic environment. Screening of spleen samples from the 25 captured *R. rattus* indicated the presence of *Bartonella* DNA in only one rat from Zaa (5.9%, 1/17) (Gundi et al., 2012). Out of 11 *Bartonella* sequences obtained from *R. norvegicus* rats, four were similar (96–100% homology) to the *Bartonella* strain 1-1C (GenBank EU551156), a *B. rochalimae*-like strain described from *R. norvegicus* in Taiwan. Interestingly, this strain was found only in *R. norvegicus,* not in *R. rattus,* although *Bartonella* prevalence was higher in the latter. Five sequences were similar with 97–100% homology to an uncultured *Bartonella* genotype previously detected in *R. rattus* in Nepal (Gundi et al., 2010). One sequence shared 100% homology with *B. queenslandensis*, a *Bartonella* species originally described in Australian rats (genera *Melomy*s and *Rattus*) (Gundi et al., 2009). In another study conducted in Kisangani, the proportion of *Bartonella*-infected rats was significantly higher in *R. rattus* (25.0%, 5/20) than in *R. norvegicus* (15.1%; 16/106) (Laudisoit et al., 2014). Two *R. rattus* and two *R. norvegicus* captured during this study in a marketplace were co-infected with *Bartonella* and *Rickettsia* species (Laudisoit et al., 2014). A strain with 100% homology with the human strain of *B. elizabethae* was reported in one of the *R. norvegicus* rats (Laudisoit et al., 2014).

Ethiopia

Meheretu et al. (2013) reported only one rat PCR-positive for *Bartonella* among 19 *R. rattus* trapped from three localities in Tigray, the northernmost region of Ethiopia. In Aroresa, where *R. rattus* comprised 46.5% in the rodent community, none of 53 tested rats was positive. The only sequence obtained from a *R. rattus* rat from Golgolnaele, Ethiopia was different from all previously described *Bartonella* species, but clustered together with genotypes found exclusively in native rodent species (*Stenocephalemys albipes* and *Arvicanthis dembeensis*) from Ethiopia (Meheretu et al., 2013).

Kenya

In a cross-sectional rat survey conducted in Kibera, an urban slum in Nairobi City, 24 of the 220 (11%) trapped rats were *Bartonella* culture-positive, including *R. norvegicus* (50%; 5/10) and *R. rattus* (60%; 19/32). In contrast, in the rural area Asembo on the northern shore of Lake Victoria, where *R. norvegicus* were absent, prevalence of *Bartonella* in *R. rattus* was lower (13%; 2/16) compared to Nairobi (Halliday et al., 2015). The high infection prevalence observed in *Rattus* trapped at the Kibera site is more similar to prevalence ranges observed in studies of Asian *Rattus* populations than to other African populations. The Kibera study in Nairobi, the Kenyan capital, clearly has more intensive connection with the seaport in Mombasa (in terms of rodent movement from other seaports) than the Asembo site located inland near Lake Victoria. Therefore, the high prevalence of *Bartonella*-infected rats reported from the Kibera site could relate to repeated introductions of *Rattus* species to this site (Halliday et al., 2015). Three zoonotic *Bartonella* species were identified in rats captured in Kibera slum area in Nairobi. They were *B. elizabethae* ($n = 7$), *B. tribocorum* ($n = 8$), and *B. queenslandensis* ($n = 4$) among 19 infected *R. rattus* and *B. elizabethae* ($n = 1$), *B. tribocorum* ($n = 3$), and *B. queenslandensis* ($n = 1$) among five infected *R. norvegicus* (Halliday et al., 2015).

Madagascar

A study conducted in Central Madagascar showed that 58.9% (93/158) of *R. rattus* rats sampled in two human communities were positive for *Bartonella* (Brook et al., 2017). They found four species: *B. elizabethae* (28; 16.9%), *B. phoceensis* (40; 23.8%), *B. rattimassiliensis* (21; 12.5%), and *B. tribocorum* (1; 0.6%) (Brook et al., 2017).

Nigeria

A study to detect *Bartonella* species in commensal rodents and their ectoparasites was conducted in town of Vom, central Nigeria. Among rats trapped there in domestic and peridomestic habitats, 36 of 121 *R. norvegicus* (29.8%), and nine of 48 *R. rattus* (18.8%) were culture-positive for *Bartonella* (Kamani et al., 2013). The prevalence of *Bartonella* DNA found in that study was

similar to the prevalence reported in rats from the Democratic Republic of Congo (Laudisoit et al., 2014). *Bartonella* strains detected in rats from Vom, a town in central Nigeria, were identical or closely related to *B. elizabethae, B. tribocorum,* and *B. grahamii* (Kamani et al., 2013). Of 36 sequences obtained from *R. norvegicus*, 26 had 98–100% similarity with *B. elizabethae* sequence. Nine of the sequences obtained from *R. norvegicus* had 97–98% similarity with *B. tribocorum* sequence, while one sequence had 98% similarity with *B. grahamii*. The *Bartonella* sequences obtained from *R. rattus* were identical to *B. elizabethae*.

South Africa

A significant difference of *Bartonella* prevalence was observed between two rat species (24% in *R. norvegicus* vs. 5% in *R. rattus*) in South Africa (Brettschneider et al., 2012). The authors of this study proposed a mathematical model explaining that the difference between these two co-occurring rat species might be due to the observed differences in flea infestation rates between these species. Trataris et al. (2012) reported *Bartonella* infections in 13% by culturing and in 25% by PCR among rats (*R. norvegicus* and *R. rattus*) sourced from a pest control company in the Ekurhuleni Metropolitan area, the East Rand region of Gauteng, South Africa. Some isolates from the rats were similar to *B. elizabethae*, while some were relatively similar to *B. thailandensis* originally described in Asian rats (Saisongkorh et al., 2009).

Uganda

A very low prevalence of *Bartonella* (1.3%; 3/228) was reported in *R. rattus* from villages of two districts of northwest Uganda, whereas a prevalence near 60% was in populations of local indigenous rodents (Billeter et al., 2014). The relatively low prevalence of infection may be due to the fact that *Rattus* rats were introduced into this area of the West Nile region relatively recently. Genotypes related to *B. elizabethae* were detected in three *R. rattus* rats (Billeter et al., 2014).

Europe

France

In contrast to numerous investigations of *Bartonella* infections in multiple sylvatic rodents in many European countries, reports of investigation of urban rats are very limited from this part of the world. Seventy-four rats (8 *R. rattus* and 66 *R. norvegicus*) were trapped in the center and suburb of the city of Marseille, France, and 20 of the *R. norvegicus,* but none of *R. rattus* were culture positive (Gundi et al., 2004). Three species of *Bartonella* were described based on characterization of the strains obtained from Norway rats from France. First, Heller et al. (1998) identified *B. tribocorum* from blood of two *R. norvegicus* rats captured near the Rhine River. Later, Gundi et al. (2004) isolated *B. rattimassiliensis* and *B. phoceensis* from rats *R. norvegicus* captured in the city of Marseille. A more recent search for *Bartonella* and various other pathogens and parasites was conducted in the urban park (Chanteraines) within Hauts-de-Seine, France (Desvars-Larrive et al., 2017). The prevalence of *Bartonella* culture-positive rats of R. norvegicus was very high (58.2%; 32/55). Surprisingly, the prevalence of *Bartonella*-DNA estimated by PCR was lower (31.4%; 27/86). The authors of this study were careful with identification of the *Bartonella* species detected in the rats, but short sequences of the obtained *pap31* amplicons were identical to *B. henselae* sequences (Desvars-Larrive et al., 2017).

Spain

The only other reported surveys of urban rats in Europe were from Spain: one from Andalusia and another one from the Canary Islands. *Bartonella* was isolated from two of ten *R. norvegicus* collected in a suburban area of Seville, Andalusia (Márquez et al., 2008). The ITS sequence analysis from *R. norvegicus* from Andalusia showed two genetically different variants (Márquez et al., 2008). The first genotype belonged to *B. tribocorum,* closely related to the strain KM2519 detected in *R. tanezumi* in China (GenBank EF202169). The second genotype was detected in 17 individuals and was relatively close to, but different from *B. elizabethae* (Márquez et al., 2008).

In the Canary Islands, *Bartonella* was found in *R. rattus* from two islands, Tenerife and La Palma (Abreu-Yanes et al., 2018). Overall, the prevalence of *Bartonella* in the rats of the Canary Islands was 14.3%, with variations between 13.8% inside houses and 26.7% in peridomestic habitats. The only two Norway rats from Tenerife were tested *Bartonella*-free. Fifteen *Bartonella* sequences identified in rats of *R. rattus* from the Canary Islands belonged to *B. tribocorum; B. queenslandensis* was in four rats, and *B. rochalimae* was recovered from two rats. Interestingly, two different *gltA* haplotypes similar to *B. elizabethae* were detected only in house mice (*Mus musculus*), but not in any rat (Abreu-Yanes et al., 2018).

Portugal

A limited number of rats from Portugal (two *R. norvegicus* from Aguas de Moura and two *R. rattus* from Porto Santo Island in Madeira Archipelago) were culture-positive for *Bartonella* (Ellis et al., 1999). The strains found in Norway rats from Portugal were relatively close to *B. tribocorum* while a strain from *R. rattus* from an island of Madeira Archipelago was evidently different (Ellis et al., 1999). Interestingly, this strain from the Portuguese island was shown later to be identical to the strains described in some *R. rattus* in Dhaka, Bangladesh and in all infected black rats in Tel Aviv, Israel (Bai et al., 2007; Harrus et al., 2009).

North America

Canada

In Vancouver, *Bartonella* species were isolated from the blood of 25.2% (102/404) of *R. norvegicus* tested (Himsworth et al., 2015). All 102 *Bartonella* cultures isolated from *R. rattus* and *R. norvegicus* from Vancouver had identical *gltA* sequences to type strain of *B. tribocorum* (Himsworth et al., 2015).

United States

Ellis et al. (1999) identified *Bartonella* species in 19.4% of *R. norvegicus* and 12% *R. rattus* collected from multiple U.S. cities. *Bartonella* infection in *R. norvegicus* varied significantly between investigated cities. In 3/9 localities, a statistically significant higher-than-expected prevalence of *Bartonella* infection in *R. norvegicus* was observed in Los Angeles California (56%), and New Orleans, Louisiana (56.4%). Seven of 66

(10.6%) *R. norvegicus* were culture-positive in Baltimore, Maryland. Rats of *R. rattus* were infected with *Bartonella* species in five of seven cities with a range of prevalence from 9 to 60% (Ellis et al., 1999). No positive rats of *R. norvegicus* were found in four cities: Atlanta, Georgia; Rockport, Indiana; Reno, Nevada; and New York City. Surprisingly, no *Bartonella*-positive rats were found in New York City among 87 rats cultured during the survey conducted by Ellis et al. (1999). In two other studies conducted in five sites within New York City, 33% (25/133) *R. norvegicus* were *Bartonella*-positive by PCR (Firth et al., 2014) and 23% (30/133) by culturing (Peterson et al., 2017). Ellis et al. (1999) described seven genotypes of *Bartonella* among 63 isolates obtained from *R. norvegicus*. The most frequently identified genotype (28/74) was 99% similar to *B. tribocorum*. Two of the *R. rattus* isolates from Louisiana were identical to one from an *R. norvegicus* captured in the same locality. Surprisingly, the 11 other isolates obtained from *R. rattus* captured in the United States were distinct from those from *R. norvegicus*. *Bartonella* strains detected in six of 11 *R. rattus* rats were identical to the genotype common for cotton rats (*Sigmodon hispidus*) from Georgia. One variant matched another cotton rat genotype from Georgia. Several other studies of urban rats in the U.S. and Canada have followed the study conducted by Ellis et al. (1999). Most genotypes identified in *R. norvegicus* from downtown Los Angeles, California were *B. tribocorum*: 101 of 106 (95.3%) culture-positive rats were infected with this particular species. In addition to *B. tribocorum*, three rats were infected with *B. queenslandensis* and two rats with *B. rochalimae* (Gundi et al., 2012). Testing blood of the same animals by PCR demonstrated presence of *B. rochalimae* in 37 (18.5%) rats and B. *queenslandensis* in four rats (2.0%) (Gundi et al., 2012). In Baltimore, Maryland, antibodies against *B. elizabethae* were detected in 34.1% of rats of *R. norvegicus* (Easterbrook et al., 2007).

Bartonella bacteria were cultured from blood of 43.5% (87/200) of *R. norvegicus* trapped in 16 sites in downtown Los Angeles, California, while *Bartonella* DNA was detected in 67.5% (135/200) of the same rats (Gundi et al., 2012). Another investigation of *Bartonella* that targeted populations of rats in California was conducted in the San Francisco Bay Area, where morphologically identified black rats represent two genetically distinct lineages that have been equated to *R. rattus* and *R. tanezumi* (Conroy et al., 2013). Of 50 of these black rats from six locations within Alameda county, California, eight *Bartonella*-positive rats were found in two locations within the City of Oakland nearly three miles apart. Interestingly, four rats from one location carried *B. tribocorum*, the bacterium dominant in rats in Los Angeles; whereas four *Bartonella*-positive rats from another location carried another bacterium, *B. coopersplainsensis,* originally described from *Rattus leucopus* in Australia (Gundi et al., 2009) and the most prevalent species in rats from New Zealand (Helan et al., 2018). In the U.S., this bacterium was found only in *R. rattus* from New Orleans (Peterson et al., 2017).

In New Orleans, Peterson et al. (2017) reported *Bartonella* infection by culture in 29 of 163 (17.8%) *R. norvegicus* and in five of 177 (2.8%) *R. rattus*. In Manhattan, New York City, 31 of 133 (23.3%) *R. norvegicus* rats tested were positive by PCR of spleen and heart tissues (Peterson et al., 2017). Peterson et al. (2017) reported significant differences in B*artonella* diversity among rats between New Orleans and New York. In New Orleans, these authors detected *B. coopersplanensis* in five roof rats. They did not detect *B. coopersplanensis* in Norway rats in either New Orleans or New York City. However, they found four other *Bartonella* species from New Orleans, and those fell within the clades corresponding to *B. rochalimae* (13 positive by PCR only), *B. elizabethae* (10 positive by culture), *B. tribocorum* (19 by culture), and *B. queenslandensis* (five positive by culture). Among 29 *Bartonella*-positive Norway rats from New York City (Manhattan), 26 rats carried *B. tribocorum*, while three rats were infected with *B. elizabethae* (Peterson et al., 2017). *Bartonella* sequences recovered from these rats were located in the same clades as *B. elizabethae, B. rochalimae*, and *B. tribocorum* (Firth et al., 2014), while sequences obtained via tissue cultures were located in the same clade as *B. elizabethae* and *B. tribocorum*.

SOUTH AMERICA

Brazil

Bartonella were isolated from 5 of 26 (19%) *R. norvegicus* rats from two of five slum areas of Salvador, the third most populous city in Brazil (Costa et al., 2014). Conducting a wide survey of various rodent species in different parts of Brazil, Gonçalves et al. (2016) detected *Bartonella* species in two *R. rattus* from Mato Grosso ($n = 3$) and Goias ($n = 4$), while rats of the same species from Ceará ($n = 12$), and Pará ($n = 8$) were free of *Bartonella*. Of 14 strains of *Bartonella* isolated from *R. norvegicus* from Salvador, all but one were identical to *B. queenslandensis*, the remaining strain was close to *B. tribocorum* (Costa et al., 2014).

Peru

Analyzing *Bartonella* species in various animals inhabiting the villages in the Huaillacayan valley, Department of Ancash, Peru, Birtles et al. (1999) isolated two strains closely resembling *B. elizabethae* from rats collected in one village. This was the first reported identification of *Bartonella* species in a commensal rat from South America. Unfortunately, the authors were unable to identify the rat species. Investigating rodents in three villages in La Convencion Province of Peru for plague and *Bartonella* species, Martin-Alonso et al. (2014) tested 24 *R. rattus* from two residential areas. All 20 rats trapped in one area (Alto Ivochote) were free of *Bartonella,* whereas one of four rats captures in another area (Yoetoni) was PCR-positive for this bacterium. One sequence obtained by Martin-Alonso et al. (2014) from *R. rattus* from La Convencion Province, along with sequences obtained from two indigenous rodents species (*H. perenensis* and *Oecomys* sp.), had 98–99% sequence similarity to a genotypic variant obtained from *Oryzomis palustris* rats in the southeastern U. S. (Kosoy et al., 1997).

Oceania

Australia

Three novel *Bartonella* species were originally described from rats of the genus *Rattus* in Australia. Those are *B. rattaustraliani* found in rats of *R. tunneyi, R. leucopus,* and *R. conatus; B.*

queenslandensis found in *R. tunneyi, R. fuscipes, R. leucopus,* and *R. conatus;* and *B. coopersplainsensis* found in *R. leucopus* (Gundi et al., 2009). The last two *Bartonella* species found in Australian rats were later discovered in rats from other continents. Two *Bartonella* species were detected in spleens of *R. rattus* from the Christmas Island, Australia (Dybing et al., 2016). Of 48 positive black rats found on this island, 28 rats carried *B. phoceensis* and eight rats carried a novel *Bartonella* genotype, potentially representing a new *Bartonella* species. Christmas Island is an Australian territory in the Indian Ocean lying in close proximity (360 km) to Jakarta, Indonesia. The authors considered the possibility that this *Bartonella* species had arrived in infected rats transported over the years on ships from nearby Indonesia (Dybing et al., 2016).

New Zealand

Using three molecular markers for identification of *Bartonella* DNA extracted from spleen samples of *R. rattus* collected in the Tongariro National Park, Helan et al. (2018) reported sequences matching *B. coopersplainsensis* and *B. henselae* from 15.4 (22/143) to 2.1% (3/143) of rats, respectively. Co-occurrence of *B. coopersplainsensis* and *B. henselae* sequences was observed in one rat. Even ff detection of prevalent *B. coopersplainsensis* was not surprising because this bacterium was described earlier in Australian rats (Gundi et al., 2009), the discovery of *B. henselae* in rats was very unexpected as this bacterium is typically found in domestic and wild cats and has never been reported previously in rats.

DISTRIBUTION OF *BARTONELLA* IN RAT POPULATIONS WITHIN URBAN TERRITORIES

Ecological factors, including size and structure of rat populations and animal movement patterns, may determine prevalence and species diversity of *Bartonella* in rats within urban territories (Firth et al., 2014; Himsworth et al., 2015; Peterson et al., 2017). During the survey conducted by Halliday et al. (2015) in Kibera, an urban slum in Nairobi, Kenya, the proportion of infected *Rattus* overall varied from 0 to 60% by trapping zone (**Figure 2**). In three zones (A, B, and D), *Mus musculus* was the dominant species and only four *Bartonella* isolates were identified in rats (**Figure 2**). Concurrently, in two other zones, *Rattus* rats dominated in rodent populations presenting 51% in zone C and 40% in zone E. In these two zones (C and E), several species of *Bartonella* were reported (**Figure 2**; Halliday et al., 2015).

According to the investigation conducted in New Orleans, Louisiana by Peterson et al. (2017), prevalence of *Bartonella* infections ranged from 0 to 97% of rats. Most *Bartonella*-positive rats (85%) were found within a single housing block of New Orleans. All *Bartonella*-positive *R. norvegicus* rats were captured at two locations, where no roof rats were present. *Bartonella*-infected *R. rattus* were captured from five locations. Though two species of rats (*R. norvegicus* and *R. rattus*) were found in four of these five locations, no *R. norvegicus* rats were *Bartonella*-positive there. A single *Bartonella*-positive *R. rattus* was captured at a location where *R. norvegicus* were absent. Of five trapping locations in New York City, *Bartonella*-positive *R. norvegicus* rats were reported in each of them; however, prevalence of infected rats at these locations ranged from 10 to 85% (Peterson et al., 2017).

Himsworth et al. (2015) demonstrated significant geographic clustering of *Bartonella*-positive rats within Vancouver, Canada. The prevalence of *B. tribocorum* varied significantly by city block, from 0 to 60.5%. Analyzing various ecological factors affecting prevalence of *B. tribocorum* in *R. norvegicus* rats from Vancouver, Rothenburger et al. (2018) noticed that the infection was significantly lower within city blocks with one or more low-rise apartment buildings compared to blocks with none. There was no significant association of the infection prevalence with rat abundance, suggesting a lack of density-dependent pathogen transmission. According to this analysis, the infection rate positively correlated with high minimum temperatures and the authors suggested that a baseline minimum temperature could be important for survival of fleas that serve are vectors for transmission of *B. tribocorum* among rats (Rothenburger et al., 2018).

Abreu-Yanes et al. (2018) defined environmental parameters related to the presence of *Bartonell*a DNA in rats in the Canary Islands. Specifically, their data suggest that occurrence of *Bartonella* on islands of this archipelago is influenced by biological and climatic conditions that vary among the islands. The probability of *Bartonella* infection in rodents in La Palma Island was four times higher compared to Tenerife Island and no *Bartonella*-positive rats were found in Lanzarote Island. A study carried out by Vicente and Gómez López (2012) showed that the flea *Stenoponia tripectinata* seem to have some preference for the conditions found on the four western islands, which include both Tenerife and La Palma, whereas this flea species was found in none of the 157 rodents from Lanzarote that belongs to the eastern group of islands. The results suggest that the ecological conditions on the Lanzarote Island are not suitable for the development of fleas *S. tripectinata*, while the ecological conditions on the La Palma Island (the most northwestern island of the archipelago) are more favorable for completing the life cycle of these fleas, which likely provide transmission of *Bartonella* infection between rats (Vicente and Gómez López, 2012).

Although *Bartonella* infections are prevalent in urban rat populations around the world and in many cities and prevalence of the infection can reach very high rates, we noted that rats in some cities and villages were not infected with *Bartonella* or the infection prevalence was quite low. At least, such situations were reported outside of Asia where we have not found reports of *Bartonella*-free populations of rats when a sufficient number of animals was tested. One of the first thoughts to explain the absence of *Bartonella* in some rat populations leads to representation of a kind of "island syndrome" wherein some parasites were absent or rare when a new rat population is established by invasion of a small number of individuals. In the regions where rats of the genus *Rattus* rarely occupy certain urban habitats, such as in areas of Europe and North America, populations of city rats are well-separated, in fact resembling

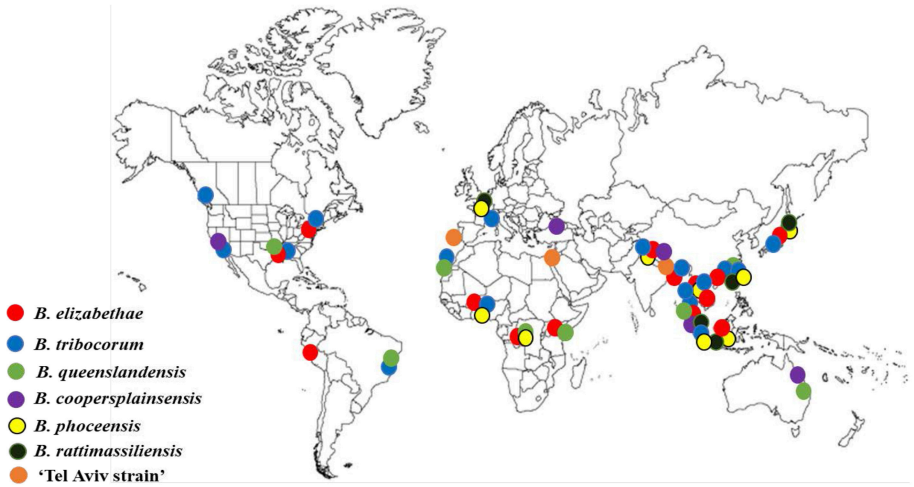

FIGURE 2 | *Bartonella* species identified in *Rattus* rats from different countries worldwide.

"island populations." This can explain the absence of *Bartonella* infections in rats from many big cities of the continental United States.

Noticeably, rats from the coastal cities on both Atlantic and Pacific sides (Los Angeles, Baltimore, New Orleans, New York, and Vancouver) carried *Bartonella*, while populations of rats in non-coastal cities were *Bartonella*-free (Ellis et al., 1999). In spite of a large number of rats investigated in Yokohama, Japan (255 *R. rattus* and 84 *R. norvegicus*), all tested animals were *Bartonella*-negative (Inoue et al., 2008). The rats of both *R. rattus* and *R. norvegicus* were *Bartonella*-free from some zones of the city of Cotonou, Benin (Martin-Alonso et al., 2016.

We admit that these observations are limited for supporting this statement. We can only speculate that continuous arrival of new rats through seaports is a crucially important factor for circulation of *Bartonella* among rats. Assuming an "island effect" on the formation of *Bartonella* communities in urban rat populations, we have to consider factors contributing to isolation of rat populations, such as time of establishing rat populations, distance and connectivity between continental rat populations and seaports where arrival of new rats is more likely to occur, rat population size, etc. (Papkou et al., 2016).

The "island effect" is not the only plausible explanation for the absence of *Bartonella* in rat populations within some urban areas and in some situations might not be the most important. When *Bartonella* bacteria are introduced into a new territory with invasive rats, the local conditions might not be favorable for the long-term circulation of the parasites within the newly established host population. Continuing an analogy borrowed from population genetics, a situation leads to the so-called "bottleneck effect" when bacteria are likely to be subjected to extreme changes in a host population. A critical issue is an availability of factors required for continuous transmission of *Bartonella* bacteria between rat hosts. A presence of appropriate flea species, particularly, the Oriental rat flea (*X. cheopis*) that can act as vectors, and the level of flea infestation, would be especially important factors for survival of *Bartonella* in rats. Unfortunately, data on the distribution of fleas in urban rat populations in the U.S. are quite sparse.

The absence of *Bartonella* infections in *Rattus* rats in some Ugandan villages and overall low prevalence in invasive rats in northwestern Uganda (Billeter et al., 2014) present another interesting situation that contrasts with numerous studies demonstrating a high prevalence of bartonellae among *Rattus* rats in Asia and in many places outside of Asia, e.g., Nairobi City. The relatively low prevalence of the infection in rats in this part of Africa may relate to relatively recent introduction of *Rattus* rats into the West Nile region. This fact is in contrast to the well-established rat populations in the cities on the coastal parts of Kenya and Tanzania, which probably have existed for millennia because of the historical dispersal of humans and their cargo via ships (Aplin et al., 2011). Despite intensive investigations, only a single rat was reported on a boat moored at Rhino Camp on the west side of the Albert Nile in the late 1930s (Hopkins, 1949; Amatre et al., 2009). Despite "fairly intensive" trapping efforts at 11 sites on land in the West Nile and West Madi regions along the west side of the Albert Nile, no *R. rattus* were captured during a survey undertaken in 1937 and 1938 by Hopkins (1949). Indeed, *R. rattus* might not have become established in the West Nile until the 1950s or even the 1960s as this rat was first identified in 1958 in the Ituri District of the DR Congo, which lies across the border from the West Nile Region (Borchert et al., 2007). This is perhaps not surprising as Hopkins (1949) suggested that *R. rattus* was first introduced to Uganda in the early Twentieth Century, a date that agrees with Delany's belief that this rat species first appeared in the country around 1910 (Delany, 1975). In this part of Africa, outsiders were restricted and movement of crops was limited until 1914 when the region became a British protectorate (Borchert et al., 2007). This likely could restrict the relocation of *R. rattus* and thereby limit the introduction of *Bartonella* infected rats. The extension of the "Kenya and Uganda Railways and Harbors" to the western Uganda in 1956 or the construction of

the bridge across the Albert Nile at Pakwach in 1969 could have lead to gradual spread of *Rattus* rats to Arua near the border with DR Congo.

THE RICHNESS OF *BARTONELLA* BACTERIAL COMMUNITIES IN ABORIGINAL RATS IN NATURAL HABITATS COMPARED TO URBAN COMMUNITIES

Provided information suggests that rat-adapted *Bartonella* species originated from Asia. The first data supporting this claim came after collection of bacteria related to *B. elizabethae* in *Rattus* rats from three districts of Yunnan province of southwestern China (Ying et al., 2002). Following this report, multiple investigations of rats in Southeast Asia (Cambodia, Laos, Thailand, and Vietnam) have also confirmed ubiquitous distribution of this infection in native rat species of the genus *Rattus* (Bai et al., 2007, 2009; Angelakis et al., 2009; Jiyipong et al., 2012). In contrast to urban rats in many countries of the world where two rat species are observed, in Asia, various species of *Rattus* occupy natural habitats from tropical lowland to mountains. For instance, the ricefield rat (*R. argentiventer*) and the Malayan field rat (*R. tiomanicus*) are common in the rice fields and plantations and the Losea rat (*R. losea*) is more typical in gardens (Kosoy et al., 2015). At the same time, only few species of rats have evidently adapted to live closely to people; specifically, *R. tanezumi* rats are common in cities, small towns, and villages.

Overall, the prevalence of *Bartonella* infections was commonly high in rats in Asia, but not higher than in urban rats in Africa or Americas. For example, Jiyipong et al. (2012) reported *Bartonella* species in 9.6% of rats in Cambodia, 11.9% in Laos, and 11.0% in Thailand, all of which are lower than the rates reported in Los Angeles, U.S. (56%) or in Nairobi, Kenya (57%). While rats of various species of *Rattus* carried *Bartonella* in natural habitats in Asia, prevalence varied between species. For example, prevalence of infected individuals has significantly varied from 3.2% in *R. exulans* to 86.4% in *R. norvegicus* in Thailand (Bai et al., 2009) or from 10% of the *R. rattus* to 66.7% of the *R. losea* in Taiwan (Hsieh et al., 2010).

The most striking difference in *Bartonella* infection between aboriginal and invasive rats was in observed diversity of the bacteria. *Bartonella* bacteria found in urban rats in Africa, Europe, and North and South Americas belonged to one or a few species or genotypes; for example, all 102 infected rats from Vancouver, Canada carried bacteria identical to each other by the *gltA*, which is a quite sensitive genetic marker (Himsworth et al., 2015). The diversity of *Bartonella* strains found in rats inhabiting natural environments in Asia was very high. Investigating rats from 17 provinces in Thailand, Bai et al. (2009) identified 23 genetic variants, which clustered with not only *B. coopersplainensis, B. elizabethae, B. phoceensis, B. rattimassiliensis,* and *B. tribocorum*, but also with numerous *Bartonella* genotypes in intermediate positions between described species or were quite different from all known species. A novel genotype of *Bartonella* with the proposed name as "Candidatus Bartonella thailandensis" was identified in rats of *Rattus surifer* from Surin, a Thai province neighboring Cambodia (Saisongkorh et al., 2009). Klangthong et al. (2015) classified DNA sequences obtained from rats in Thailand into eight different cladograms. *Bartonella* sequences obtained from rats of several *Rattus* species from Southeast Asia represented over 40 different genetic variants and clustered into nine lineages (Jiyipong et al., 2012). All described rat-adapted *Bartonella* species were identified in rats (*R. argentiventer*, *R. tanezumi*, *R. norvegicus*, and *Bandicota indica*) from the Mekong Delta in Vietnam (Loan et al., 2015). The prevalence of *Bartonella* infection among rats trapped in farms, filed, and forest was 22.4%, much higher than the infection prevalence in rats that were purchased in city markets (8.7%). The highest prevalence was found in *R. tanezumi* (49.2%), followed by *R. norvegicus* (20.7%). No *Bartonella* was found in *R. exulans.* The species isolated from these rats were *B. rattimassiliensis*, *B. tribocorum*, *B. elisabethae, B. coopersplainensis,* and *B. queenslandensis* (corresponding to 43.8, 21.9, 18.8, 9.4, and 6.3% of 32 *Bartonella*-infected rats. Two species (*B. rattimassiliensis* and *B. coopersplainensis*) were identified in *R. tanezumi* only, while all other species of *Bartonella* were detected at least in two rat species (Loan et al., 2015). The prevalence of *Bartonella* species in rats from rural parts of Vietnam (Mekong Delta) was significantly lower than in Saigon Port, but the diversity of the species was evidently higher.

PHYLOGENETIC RECONSTRUCTION OF GLOBAL DISSEMINATION OF *BARTONELLA* BY RATS

A comparison of *Bartonella* bacterial communities between aboriginal and invasive rats of the genus *Rattus* allows an investigation of the roles played by rats as carriers of these bacteria comparing the diversity of *Bartonella* genotypes in rats between Southeast Asia where presumably original diversification happened and other parts of the world where only two rat species were relatively recently introduced. Hayman et al. (2013) analyzed variations of one genetic locus (the *gltA* as the most widely used molecular marker for differentiation of *Bartonella* species) of 191 strains of rat-associated bartonellae from 17 countries. The phylogeographic analysis supported the hypotheses that *Bartonella* species likely originated in Southeast Asia. The analysis has also highlighted the role of *R. rattus* in disseminating *Bartonella* bacteria to other continents. Black rats have invaded most countries of the world with main introductions that happened through several commensalism events (Aplin et al., 2011). Furthermore, the phylogenetic analysis conducted by Hayman et al. (2013) demonstrated that diversification of species belonging to the *B. elizabethae* species complex occurred in Southeast Asia before some of the species belonging to this complex were transmitted to other geographic regions. Importantly, their analysis suggests that there were multiple disseminations of these bacteria within Asia and numerous introductions from Asia to other parts of the world. This conclusion is based on identification of several

major clades of *Bartonella* strains of Southeast Asian origin that dispersed globally.

Specifically, *Bartonella* strains obtained from rats of the genus *Rattus* from different continents and countries were grouped into six major clades that presumably originated in Southeast Asia. Of those, one clade (A) is distributed globally with strains found in most regions, but not in Central Africa. Likely, *R. norvegicus* play the leading role in distributing *Bartonella* species belonging to this clade. The analysis also suggests that the process of bacterial dispersal in this species clade is still continuous. Strains belonging to another clade (B) were detected only in Asia and Western Europe. The strains belonging to the third clade (C) dispersed to countries of Africa, North and South America. Strains grouped into the D are found in Africa and North America, besides Asia. Clade E has limited geographic spread, with only a Eurasian distribution. Finally, the strains combined into the clade F are distributed across Pacific and were detected in East Asia, Australia, and western part of North America (Hayman et al., 2013).

BARTONELLA DNA IN RAT ECTOPARASITES

Multiple field observations and limited experimental studies support the major role of rat fleas in transmission of bartonellae among rats. Gutiérrez et al. (2015) highlighted the importance of the level of the flea's host specificity, flea exchange between rodents, and flea abundance for success of transmission of *Bartonella* bacteria. The host specificity of some flea species, e.g., *X. cheopis*, may influence the acquisition or the restriction of specific *Bartonella* species and strains to rats. There are many reports of the presence of *Bartonella* DNA in ectoparasites collected from *Rattus* rats. *Bartonella* DNA was detected in 59.1% of 193 *Xenopsylla cheopis* fleas collected from 62 *Rattus* rats (*R. exulans, R. norvegicus*, and *R. rattus*) captured in Khon Kaen, the northeastern province of Thailand. Sequence analysis of DNA present in rat fleas from this area demonstrated the presence of *Bartonella* species similar to *B. elizabethae*, *B. rattimassiliensis*, *B. rochalimae*, and *B. tribocorum* (Billeter et al., 2013). Another study in Thailand targeting rats and their ectoparasites in villages from all regions of the country indicated that the prevalence of *Bartonella* DNA varied substantially depending on ectoparasite species (Klangthong et al., 2015). Of the multiple arthropods screened during this study, the highest prevalence of *Bartonella* DNA was in louse (*Polyplax* and *Hoplopleura*, 57.1%) and flea (*X. cheopis*, 25.8%) pools. Only few positive samples were found in pools of mites (*Leptotrombidium* and *Ascoschoengastia*, prevalence 1.7%) and ticks (*Haemaphysalis* species, prevalence 3.5%). Most identified sequences found in arthropods have been found in rats and belong to the *B. elizabethae* species complex. Eight sequences of *B. tribocorum* were detected from six flea pools. One genotype identified as *B. queenslandensis* (99.6% identity) was found in a flea pool. One flea pool was positive for *Bartonella* with genotype being not reported in rats, but still closely related to *B. tribocorum* (96.8 % identity). Some identified *Bartonella* sequences from tick and louse pools shared close similarity with *B. coopersplainsensis.* Interestingly, *B. phoceensis* DNA was detected solely from ectoparasites (louse, mite, and tick pools) (Klangthong et al., 2015). Fleas obtained from rats in Taiwan harbored DNA of several *Bartonella* species (Tsai et al., 2010). *Bartonella* DNA detected in eight lice (*Polyplax*) obtained from five *Bartonella*-positive *R. norvegicus* from Taiwan was identified as *B. phoceensis*. The authors noticed that fleas collected from adult rats (77.1%) more likely *Bartonella*-positive than fleas collected from juvenile rats (42.3%).

Laudisoit et al. (2014) reported a high prevalence of *Bartonella* DNA in rat fleas from Kisangani, D. R. Congo. *Bartonella* genotypes detected pools of fleas *X. cheopis*, ticks *R. sanguineus*, and mites *Haemolaelaps* from Nigeria were identical or similar *B. elizabethae* (97–100% similarity), but a genotype found in a flea *Ctenophthalmus* pool was *B. tribocorum* (97% similarity) (Kamani et al., 2013). Nine of 12 genetic variants detected in rat fleas in Uganda belonged to the *B. elizabethae* species complex (Bai et al., 2017). In Madagascar, *B. elizabethae* was found in fleas of *Synopsyllus fonquerniei* and *X. cheopis,* while *B. phoceensis* and *B. rattimassiliensis* were found in sucking lice of the genus *Polyplax* (Brook et al., 2017).

In New York City, *Bartonella* DNA recovered from Oriental rat fleas collected from Norway rats belonged to three *Bartonella* species. The most common sequences clustered with *B. tribocorum*, while sequences related to *B. elizabethae* and *B. rochalimae* were less common in fleas (Frye et al., 2015). There are more reports of *Bartonella* DNA detected in rat blood. The main message that we can deliver from these studies is that the range of *Bartonella* genotypes found in ectoparasites, especially in fleas matches very much the spectrum of bacterial species found in rats. This contrasts with some observations made during investigations of sylvatic rodent communities when a considerable discordance between genotypes of *Bartonella* obtained from several 'rodent host-ectoparasite' pairs was reported (Abbot et al., 2007). A recent experimental study conducted by McKee et al. (2018) supported vector capacity of Oriental rat fleas (*X. cheopis*) for transmission of a rat-adapted *Bartonella* species. Specifically, this experiment demonstrated acquisition of *B. elizabethae* by experimentally exposed rat fleas and excretion of *Bartonella* DNA in flea feces over several days (McKee et al., 2018).

RAT-ASSOCIATED BARTONELLOSIS IN HUMANS

Pathogens carried by Norway and black rats can lead to significant morbidity and mortality in people around the world (Himsworth et al., 2013). The first species of rat-associated *Bartonella* proven to be a human pathogen was *B. elizabethae* (at that time described as *Rochalimaea elizabethae*) (Daly et al., 1993). This bacterium was isolated from blood of a 31-year old male patient with endocarditis admitted to the Saint Elizabeth Hospital in Massachusetts, U.S. The patient had no history of exposure to cats or other pets or intravenous drug use. A source of the infection remained mysterious until Ellis et al. (1999) found relatively similar bacteria in rats from the U.S. Ying et al. (2002)

reported a variety of similar bacteria in rats in Southern China and, finally, an identical bacterium was found to be prevalent in rats from Vietnam (Loan et al., 2015). Later, an identical bacterium was identified in a febrile patient in Bangladesh (Faruque et al., 2017).

The strains identical or closely similar to rat-adapted *Bartonella* species, including *B. elizabethae, B. tribocorum,* and *B. rattimassiliensis* were identified in blood clots from eight febrile patients from two Thai provinces, Chiang Rai and Khon Kaen (Kosoy et al., 2010). These genotypes represented more than one-half of the *Bartonella* genotypes identified in human patients with fever of unknown etiology enrolled into this study. Importantly, some genotypes identified in rats from Los Angeles showed 98.8% similarity to the isolate obtained from a Thai patient (GenBank accession number GQ225706) (Gundi et al., 2012). Moreover, the strain of *B. tribocorum* identified in a Thai patient was identical to a *Bartonella* sequence detected in *X. cheopis* fleas collected from *R. norvegicus* rats in Los Angeles, California (Billeter et al., 2011). More recently, a strain of *B. tribocorum* was cultured with a bacteremia level of 60 colonies per 1ml from the blood of a 64-year old male patient with complaints of fatigue, muscle pain, and headache in France (Vayssier-Taussat et al., 2016).

Another species of *Bartonella (B. rochalimae),* though not specific for rats, was found in *Rattus* rats and their fleas from Asia and America (Billeter et al., 2011; Gundi et al., 2012). This bacterium was originally described as a human pathogen when it apparently caused fever and splenomegaly in a U.S. patient who became ill after traveling to Peru (Eremeeva et al., 2007). This bacterium was found in dogs and many wild mammalian species (foxes, rats, shrews, gerbils, and raccoons), suggesting that multiple reservoirs may be involved in its maintenance (Bai et al., 2016).

From our standpoint, the most intriguing and convincing case was reported from Tbilisi, the capital of the country of Georgia, where an 18-year old woman was admitted to hospital with a 2-week history of malaise, fever, and severe lymphadenopathy (Kandelaki et al., 2016). The patient lived in a residential area within Tbilisi and had not recently traveled outside the city. Based on lymphadenopathy and some other clinical manifestations, clinicians suspected cat scratch disease (CSD) although the patient denied any contact with cats. The clinical specimens were sent to the laboratory and results proved that indeed the bacterium found in samples from the patient belonged to the genus *Bartonella.* However, analyses that are more precise demonstrated that the strain was not *B. henselae*, the agent of CSD, but belonged to the *B. elizabethae* species complex. Thorough phylogenetic analysis involving seven molecular targets demonstrated that the bacterium had a divergence of 3.4% from *B. elizabethae* and 5.6% from *B. tribocorum.* Most importantly, this strain was identical to the Tel Aviv strain of *Bartonella*, which is prevalent and the only strain identified among *R. rattus* rats captured in Tel Aviv, Israel (Harrus et al., 2009).

The results of several serological surveys supported a potential exposure of people to rat-adapted *Bartonella* species. A survey of 630 drug users conducted in Baltimore, Maryland, reported seroprevalence of antibodies to rat-specific *B. elizabethae* (33%), 3-fold higher than prevalence to the cat-specific *B. henselae* (11%) or louse-transmitted *B. quintana* (10%) (Comer et al., 1996). A similar survey conducted in Central and East Harlem in New York City showed an even higher prevalence of seroreactivity to *B. elizabethae* (46%) compared to antibody positivity observed to *B. henselae* (10%) and *B. quintana* (2%) (Comer et al., 2001). A study of homeless people in Stockholm, Sweden reported high seroprevalence (52%) to *B. elizabethae* (Ehrenborg et al., 2008). McVea et al. (2018) reported exposure to rat-associated *Bartonella* species among intravenous drug users in an impoverished neighborhood of Vancouver, Canada. A retrospective serological survey of archived specimens indicated that *Bartonella* antibodies are prevalent among febrile patients in the Kathmandu Valley of Nepal (Myint et al., 2011). When 11 cases with high titers were compared to eight different *Bartonella* antigens, the highest titers (ranged from 1:256 to 1:2,048) reported in three patients were against the antigen of *B. elizabethae*.

THE MOVEMENT OF *BARTONELLA* BY RATS FROM SOUTHEAST ASIA TO URBAN CENTERS IN OTHER PARTS OF THE WORLD

A high diversity of *Bartonella* species and strains on the one side and association of specific *Bartonella* species with mammalian hosts on the other, in our case with rats of the genus *Rattus*, provide an opportunity for reconstructing the movement of these bacteria from the jungles of Southeast Asia to cities on all continents except for Antarctica. The studies of *Bartonella* strains associated with the rats of genera *Rattus* and *Bandicota* demonstrated that these bacteria cluster into a separate phylogenetic lineage (Heller et al., 1998; Ellis et al., 1999; Ying et al., 2002; Castle et al., 2004; Gundi et al., 2004, 2009). These *Bartonella* species likely originated in Southeast Asia and subsequently dispersed from Asia with *Rattus* rats because of human activity. Later these bacteria became common and widespread in urban and peridomestic environments around the world (Childs et al., 1999; Ellis et al., 1999).

We have to distinguish roles played by rats as hosts of *Bartonella* within the lands where they had originally diversified from rats that have been translocated in recent human history. Data supporting the hypothesis of the Old World origin of *Rattus* rat-associated *Bartonella* species include the widespread occurrence of genetically related isolates of *Bartonella* species in *R. norvegicus* from Portugal, the United States, and South America (Buffet et al., 2013). On the other hand, there is an evident difference between the *Bartonella* isolates obtained from rats and from indigenous rodents of America (Ellis et al., 1999). The first evidence *Bartonella* genotypes from Southeastern Asia being related to *B. elizabethae* came from identification of the high diversity of *Bartonella* in *R. tanezumi* rats in several cities of southern China and in lesser bandicoot rats (*Bandicota bengalensis*) and black rats (*R. rattus*) in Dhaka, Bangladesh (Ying et al., 2002; Bai et al., 2007). A few of the multitude of genotypes

found in these rats were identical to sequences of cultures from *Rattus* rats found in France, Portugal, and the United States.

We propose to consider a "source-sink" ecological model developed in the field of population ecology of animals and plants (Pulliam, 1988) for comprehending the differences described earlier in this article. According to the original scheme, rat populations distributed across source habitats within the native range in Asia ("source") are self-sustaining; while the rat populations introduced to other continents ("sink") can be maintained continuously only by immigration of rats from natural habitats. Assuming the role of aboriginal rat populations as "sources" and the role of commensal rats in the continents where rats were introduced relatively recently as "sinks," we can propose one more component for inclusion into this conceptual model. The assemblage of *Bartonella* strains in rats inhabiting big cities in Asia is commonly less diverse compared to populations of rats in native habitats and rural areas within the range of natural origin, but more diverse than in cities of other continents. These communities represent intermediate positions in the "source-sink" model. For example, the number of rat species in Bangkok, Ho Chi Minh City, Yangon, and others is restricted to only two commensal rat species common to urban areas in Africa, Americas, and Europe; but have a reduced diversity compared to communities observed in the forests or fields in Southeast Asia outside cities. The reduced number of rats in big Asian cities can explain the intermediate rate of *Bartonella* circulated within these rat populations. For example, the diversity of *Bartonella* species observed in rats from Dhaka, Bangladesh (Bai et al., 2007) was higher than in rats from cities in Americas and Europe (Gundi et al., 2012), but lower than in natural and agricultural settings in Thailand (Bai et al., 2009). Although all described *Rattus* rat-associated species of *Bartonella* have a worldwide distribution, the diversity of *Bartonella* genotypes in rats from natural habitats of southeastern Asia is much higher compared to a number of strains reported in all cities of the world outside Asia.

In a number of studies, *Bartonella* infection prevalence was higher in *R. norvegicus* compared to *R. rattus* (Ellis et al., 1999; Hsieh et al., 2010; Martin-Alonso et al., 2016). In some situations, this difference can be explained by the load of ectoparasites carried by these rats, but likely this is not the sole explanation. Brettschneider et al. (2012) noticed a similar effect and argued that more detailed biological research on *Bartonella* infections is needed to explain such observations. Based on comparative phylogeography of invasive rats in the United States, Lack et al. (2013) came to conclusion that rats of *R. norvegicus* may contribute to a greater diversity of pathogens from various international sources and spread them across the U.S. compared to *R. rattus*. Their premise is based the data suggesting that gene flow among populations was higher for the Norway rats compared to *R. rattus* (Lack et al., 2013). In addition, their analyses support their hypothesis that *R. norvegicus* rats invade both Atlantic and Pacific coasts of the U.S. and likely from different points of their origin (Lack et al., 2013). A comparison of *Bartonella* observed in both *R. norvegicus* and *R. rattus* in the US cities (Ellis et al., 1999) and phylogenetic analysis of *Bartonella* isolates conducted by Hayman et al. (2013) support this supposition.

CONCLUSIONS

Bartonella species, being a highly prevalent and extremely diverse group of bacteria, are excellent sentinel organisms for evaluating the transoceanic and intra-continental movement of the pathogens by rats of the genus *Rattus*. The analyses described in this article confirmed the role of human-mediated distribution of invasive rat species in dissemination of rat-adapted parasites. Intensive collections and characterization of the *Bartonella* strains recovered from *Rattus* rats allowed the demonstration of the global dissemination of such strains from Asia to Africa, Australia, Europe, and finally to the Americas. Phylogenetic analyses of rat-adapted strains represent an interesting model for investigating pathogen-host coevolution. The interesting question remains how introduction of specialized parasites introduced via invasive rodent hosts can alter parasite community dynamics. Finally, the accumulation of reports of human cases associated with rat-borne *Bartonella* species has increased concern about public health consequences of the global distribution of these bacteria and their introduction to urban centers.

AUTHOR CONTRIBUTIONS

MK and YB contributed conception and design of the study, organized the database, wrote the manuscript.

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REFERENCES

Abbot, P., Aviles, A. E., Eller, L., and Durden, L. A. (2007). Mixed infections, cryptic diversity, and vector-borne pathogens: evidence from *Polygenis* fleas and *Bartonella* species. *Appl. Environ. Microbiol.* 73, 6045–6052. doi: 10.1128/AEM.00228-07

Abreu-Yanes, E., Martin-Alonso, A., Martin-Carrillo, N., Livia, K. G., and Marrero-Gagliardi, A., Valladares, B., et al. (2018). *Bartonella* in Rodents and ectoparasites in the Canary Islands, Spain: new insights into host-vector-pathogen relationships. *Microb. Ecol.* 75, 264–273. doi: 10.1007/s00248-017-1022-y

Amatre, G., Babi, N., Enscore, R. E., Ogen-Odoi, A., Atiku, L. A., Akol, A., et al. (2009). Flea diversity and infestation prevalence on rodents in a plague-endemic region of Uganda. *Am. J. Trop. Med. Hyg.* 81, 718–724. doi: 10.4269/ajtmh.2009.09-0104

Angelakis, E., Khamphoukeo, K., Grice, D., Newton, P. N., Roux, V., Aplin, K., et al. (2009). Molecular detection of *Bartonella* species in rodents from the Lao PDR. *Clin. Microbiol. Infect.* 2, 95–97. doi: 10.1111/j.1469-0691.2008.02177.x

Aplin, K. P., Suzuki, H., Chinen, A. A., Chesser, R. T., Ten Have, J., Donnellan, S. C., et al. (2011). Multiple geographic origins of commensalism and complex dispersal history of black rats. *PLoS ONE* 6:e26357. doi: 10.1371/journal.pone.0026357

Bai, Y., Gilbert, A., Fox, K., Osikowicz, L., and Kosoy, M. (2016). *Bartonella rochalimae* and *B. vinsonii subsp.* berkhoffii in wild carnivores from Colorado, USA. *J Wildl Dis*. 52, 844–849. doi: 10.7589/2016-01-015

Bai, Y., Kosoy, M. Y., Lerdthusnee, K., Peruski, L. F., and Richardson, J. H. (2009). Prevalence and genetic heterogeneity of *Bartonella* strains cultured from rodents from 17 provinces in Thailand. *Am. J. Trop. Med. Hyg.* 81, 811–816. doi: 10.4269/ajtmh.2009.09-0294

Bai, Y., Montgomery, S. P., Sheff, K. W., Chowdhury, M. A., Breiman, R. F., Kabeya, H., et al. (2007). *Bartonella* strains in small mammals from Dhaka, Bangladesh, related to *Bartonella* in America and Europe. *Am. J. Trop. Med. Hyg.* 77, 567–570. doi: 10.4269/ajtmh.2007.77.567

Bai, Y., Osikowicz, L. M., Kosoy, M. Y., Eisen, R. J., Atiku, L. A., and Mpanga, J. T. (2017). Comparison of zoonotic bacterial agents in fleas collected from small mammals or host-seeking fleas from a Ugandan region where plague is endemic. *mSphere*. 2:e00402–17. doi: 10.1128/mSphere.00402-17

Battersby, S., Hirschhorn, R. B., and Amman, B. R. (2008). "Commensal rodents," in *Public Health Significance of Urban Pests,* eds. X. Bonnefoy, H. Kampen, K. Sweeney, and World Health Organization (Copenhagen: World Health Organization), 387–419.

Billeter, S. A., Borchert, J. N., Atiku, L. A., Mpanga, J. T., Gage, K. L., and Kosoy, M. Y. (2014). *Bartonella* species in invasive rats and indigenous rodents from Uganda. *Vector Borne Zoonotic Dis.* 14, 182–188. doi: 10.1089/vbz.2013.1375

Billeter, S. A., Colton, L., Sangmaneedet, S., Suksawat, F., Evans, B. P., and Kosoy, M. Y. (2013). Molecular detection and identification of *Bartonella* species in rat fleas from northeastern Thailand. *Am. J. Trop. Med. Hyg.* 89, 462–465. doi: 10.4269/ajtmh.12-0483

Billeter, S. A., Gundi, V. A., Rood, M. P., and Kosoy, M. Y. (2011). Molecular detection and identification of Bartonella species in *Xenopsylla cheopis* fleas (Siphonaptera: Pulicidae) collected from *Rattus norvegicus* rats in Los Angeles, California. *Appl. Environ. Microbiol.* 77, 7850–7852. doi: 10.1128/AEM.06012-11

Birtles, R. J. (2005). Bartonellae as elegant hemotropic parasites. *Ann. N. Y. Acad. Sci.* 1063, 270–279. doi: 10.1196/annals.1355.044

Birtles, R. J., Canales, J., Ventosilla, P., Alvarez, E., Guerra, H., Llanos-Cuentas, A., et al. (1999). Survey of *Bartonella* species infecting intradomicillary animals in the Huayllacallán Valley, Ancash, Peru, a region endemic for human bartonellosis. *Am. J. Trop. Med. Hyg.* 60, 799–805. doi: 10.4269/ajtmh.1999.60.799

Bitam, I., Rolain, J. M., Nicolas, V., Tsai, Y. L., Parola, P., Gundi, V. A., et al. (2012). A multi-gene analysis of diversity of *Bartonella* detected in fleas from Algeria. *Comp. Immunol. Microbiol. Infect. Dis.* 35, 71–76. doi: 10.1016/j.cimid.2011.11.002

Borchert, J. N., Mach, J. J., and Linder, T. J. (2007). "Invasive Rats and Bubonic Plague in Northwest Uganda," in *Managing Vertebrate Invasive Species: Proceedings of an International Symposium*, eds G. W. Witmer, W. C. Pitt, and K. A. Fagerstone (Fort Collins: USDA/APHIS Wildlife Services, National Wildlife Research Center), 283–293.

Bradley, C. A., and Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends Ecol. Evol.* 22, 95–102. doi: 10.1016/j.tree.2006.11.001

Brettschneider, H., Anguelov, R., Chimimba, C. T., and Bastos, A. D. (2012). A mathematical epidemiological model of gram-negative *Bartonella* bacteria: does differential ectoparasite load fully explain the differences in infection prevalence of *Rattus rattus* and *Rattus norvegicus*? *J. Biol. Dyn.* 6, 763–781. doi: 10.1080/17513758.2012.705906

Brook, C. E., Bai, Y., Yu, E. O., Ranaivoson, H. C., Shin, H., Dobson, A. P., et al. (2017). Elucidating transmission dynamics and host-parasite-vector relationships for rodent-borne *Bartonella* spp. in Madagascar. *Epidemics* 20, 56–66. doi: 10.1016/j.epidem.2017.03.004

Buffet, J. P., Kosoy, M., and Vayssier-Taussat, M. (2013). Natural history of *Bartonella*-infecting rodents in light of new knowledge on genomics, diversity and evolution. *Future Microbiol.* 8, 1117–1128. doi: 10.2217/fmb.13.77

Castle, K. T., Kosoy, M., Lerdthusnee, K., Phelan, L., Bai, Y., Gage, K. L., et al. (2004). Prevalence and diversity of *Bartonella* in rodents of northern Thailand: a comparison with Bartonella in rodents from southern China. *Am. J. Trop. Med. Hyg.* 70, 429–433.

Çelebi, C., Karagöz, A., Öktem, M. A., Carhan, A., and Ozkazanc, N. K., Babur, C. et al. (2015). *Bartonella* species in wild small mammals in Western Black Sea Region of Turkey. *Vet. Fakültesi dergisi* 62, 183–187. doi: 10.1501/Vetfak_0000002678

Cevidanes, A., Altet, L., Chirife, A. D., Proboste, T., and Millán, J. (2017). Drivers of *Bartonella* infection in micromammals and their fleas in a Mediterranean peri-urban area. *Vet. Microbiol.* 203, 181–188. doi: 10.1016/j.vetmic.2017.03.023

Childs, J. E., Ellis, B. A., Nicholson, W. L., Kosoy, M., and Sumner, J. W. (1999). Shared vector-borne zoonoses of the Old World and New World: home grown or translocated? *Schweiz. Med. Wochenschr*. 129, 1099–1105.

Childs, J. E., Glass, G. E., Ksiazek, T. G., Rossi, C. A., Oro, J. G., and Leduc, J. W. (1991). Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. *Am. J. Trop. Med. Hyg.* 44, 117–121. doi: 10.4269/ajtmh.1991.44.117

Comer, J. A., Diaz, T., Vlahov, D., Monterroso, E., and Childs, J. E. (2001). Evidence of rodent-associated *Bartonella* and *Rickettsia* infections among intravenous drug users from Central and East Harlem, New York City. *Am. J. Trop. Med. Hyg.* 65, 855–860. doi: 10.4269/ajtmh.2001.65.855

Comer, J. A., Flynn, C., Regnery, R. L., Vlahov, D., and Childs, J. E. (1996). Antibodies to *Bartonella* species in inner-city intravenous drug users in Baltimore, Md. *Arch. Intern. Med.* 156, 2491–2495. doi: 10.1001/archinte.1996.00440200111014

Conroy, C. J., Rowe, K. C., Rowe, K. M., Kamath, P. L., Aplin, K. P., Lucia Hui, L., et al. (2013). Cryptic genetic diversity in *Rattus* of the San Francisco Bay region, California. *Biol. Invasions* 15, 741–758. doi: 10.1007/s10530-012-0323-9

Costa, F., Porter, F. H., Rodrigues, G., Farias, H., de Faria, M. T., Wunder, E. A., et al.(2014). Infections by *Leptospira interrogans*, Seoul virus, and *Bartonella* spp. among Norway rats *(Rattus norvegicus*) from the urban slum environment in Brazil. *Vector Borne Zoonotic Dis*. 14, 33–40. doi: 10.1089/vbz.2013.1378

Crowl, T. A., Crist, T. O., Parmenter, R. R., Belovsky, G., and Lugo, A. E. (2008). The spread of invasive species and infectious disease as drivers of ecosystem change. *Front. Ecol. Environ.* 6, 238–246. doi: 10.1890/070151

Daly, J. S., Worthington, M. G., Brenner, D. J., Moss, C. W., Hollis, D. G., Weyant, R. S., et al. (1993). *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. *J. Clin. Microbiol.* 31, 872–881.

Delany, M. J. (1975). *Rodents of Uganda*. (London: British Museum Press), 173.

Desvars-Larrive, A., Pascal, M., Gasqui, P., Cosson, J. F., Benoît, E., Lattard, V., et al. (2017). Population genetics, community of parasites, and resistance to rodenticides in an urban brown rat (*Rattus norvegicus*) population. *PLoS ONE* 12:e0184015. doi: 10.1371/journal.pone.0184015

Dybing, N. A., Jacobson, C., Irwin, P., Algar, D., and Adams, P. J. (2016). *Bartonella* species identified in rodent and feline hosts from island and Mainland Western Australia. *Vector Borne Zoonotic Dis.* 16, 238–244. doi: 10.1089/vbz.2015.1902

Easterbrook, J. D., Kaplan, J. B., Vanasco, N. B., Reeves, W. K., Purcell, R. H., Kosoy, M. Y., et al. (2007). A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol. Infect.* 135, 1192–1199. doi: 10.1017/S0950268806007746

Easterbrook, J. D., Shields, T., Klein, S. L., Glass, G. E. (2005). Norway rat population in Baltimore, Maryland, 2004. *Vector Borne Zoonotic Dis.* 5, 296–299. doi: 10.1089/vbz.2005.5.296

Ehrenborg, C., Byström, R., Hjelm, E., Friman, G., and Holmberg, M. (2008). High *Bartonella* spp. seroprevalence in a Swedish homeless population but no evidence of trench fever. *Scand. J. Infect. Dis.* 40, 208–215. doi: 10.1080/00365540701632972

Ellis, B. A., Regnery, R. L., Beati, L., Bacellar, F., Rood, M., Glass, G. G., et al. (1999). Rats of the genus *Rattus* are reservoir hosts for pathogenic *Bartonella* species: an Old World origin for a New World disease? *J. Infect. Dis.* 180, 220–224.

Eremeeva, M. E., Gerns, H. L., Lydy, S. L., Goo, J. S., Ryan, E. T., Mathew, S. S., et al. (2007). Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. *N. Engl. J. Med.* 356, 2381–2387. doi: 10.1056/NEJMoa065987

Faruque, L. I., Zaman, R. U., Gurley, E. S., Massung, R. F., Alamgir, A. S., Galloway, R. L., et al. (2017). Prevalence and clinical presentation of *Rickettsia, Coxiella, Leptospira, Bartonella* and chikungunya virus infections among hospital-based febrile patients from December 2008 to November 2009 in Bangladesh. *BMC Infect. Dis.* 17:141. doi: 10.1186/s12879-017-2239-6

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P., et al. (2014). Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. *MBio* 5, 1–16. doi: 10.1128/mBio.01933-14

Frye, M. J., Firth, C., Bhat, M., Firth, M. A., Che, X., and Lee, D. (2015). Preliminary survey of ectoparasites and associated pathogens from Norway rats in New York City. *J. Med. Entomol.* 52, 253–259. doi: 10.1093/jme/tjv014

Gardner-Santana, L. C., Norris, D. E., Fornadel, C. M., Hinson, C. M., Klein, S. L., and Glass, G. E. (2009). Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*). *Mol. Ecol.* 8, 2766–2778. doi: 10.1111/j.1365-294X.2009.04232.x

Gonçalves, L. R., Favacho, A. R., Roque, A. L., Mendes, N. S., Fidelis, O. L., Benevenute, J. L., et al. (2016). Association of *Bartonella* species with wild and synanthropic rodents in different Brazilian biomes. *Appl. Environ. Microbiol.* 82, 7154–7164. doi: 10.1128/AEM.02447-16

Grimmond, S. U. E. (2007). Urbanization and global environmental change: local effects of urban warming. *Geograph. J.* 173, 83–88. doi: 10.1111/j.1475-4959.2007.232_3.x

Gundi, V. A., Billeter, S. A., Rood, M. P., and Kosoy, M. Y. (2012). *Bartonella* spp. in rats and zoonoses, Los Angeles, California, USA. *Emerg. Infect. Dis.* 18, 631–633. doi: 10.3201/eid1804.110816

Gundi, V. A., Kosoy, M. Y., Myint, K. S., Shrestha, S. K., Shrestha, M. P., Pavlin, J. A., et al. (2010). Prevalence and genetic diversity of *Bartonella* species detected in different tissues of small mammals in Nepal. *Appl. Environ. Microbiol.* 76, 8247–8254. doi: 10.1128/AEM.01180-10

Gundi, V. A. K. B., Davoust, B., Khamis, A., Boni, M., Raoult, D., and La Scola, B. (2004). Isolation of *Bartonella rattimassiliensis* sp. nov. and *Bartonella phoceensis* sp. nov. from European *Rattus norvegicus*. *J Clin Microbiol.* 42: 3816–3818. doi: 10.1128/JCM.42.8.3816-3818.2004

Gundi, V. A. K. B., Taylor, C., Raoult, D., and La Scola, B. (2009). Bartonella rattaustraliani sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. *Int. J. Syst. Evol. Microbiol.* 59, 2956–2961. doi: 10.1099/ijs.0.002865-0

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I. S., and Harrus, S. (2015). *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector Borne Zoonotic Dis.* 15, 27–39. doi: 10.1089/vbz.2014.1606

Halliday, J. E., Knobel, D. L., Agwanda, B., Bai, Y., Breiman, R. F., Cleaveland, S., et al. (2015). Prevalence and diversity of small mammal-associated *Bartonella* species in rural and urban Kenya. *PLoS Negl. Trop. Dis.* 9:e0003608. doi: 10.1371/journal.pntd.0003608

Harrus, S., Bar-Gal, G. K., Golan, A., Elazari-Volcani, R., Kosoy, M. Y., Morick, D., et al. (2009). Isolation and genetic characterization of a *Bartonella* strain closely related to Bartonella *tribocorum* and *Bartonella elizabethae* in Israeli commensal rats. *Am. J. Trop. Med. Hyg.* 81, 55–58. doi: 10.4269/ajtmh.2009.81.55

Hayman, D. T., McDonald, K. D., and Kosoy, M. Y. (2013). Evolutionary history of rat-borne *Bartonella*: the importance of commensal rats in the dissemination of bacterial infections globally. *Ecol. Evol.* 3, 3195–3203. doi: 10.1002/ece3.702

Helan, J. V. G., Grinberg, A., Gedye, K., Potter, M. A., and Harrus, S. (2018). Molecular detection of *Bartonella coopersplainsensis* and *B. henselae* in rats from New Zealand. *N. Z. Vet. J.* 66, 257–260. doi: 10.1080/00480169.2018.1483781

Heller, R., Riegel, P., Hansmann, Y., Delacour, G., Bermond, D., Dehio, C., et al. (1998). *Bartonella tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. *Int. J. Syst. Bacteriol.* 48 (Pt 4), 1333–1339. doi: 10.1099/00207713-48-4-1333

Himsworth, C. G., Bai, Y., Kosoy, M. Y., Wood, H., DiBernardo, A., Lindsay, R., et al. (2015). An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: is pathogen presence a reflection of global and local rat population structure? *Vector Borne Zoonotic Dis.* 15, 21–26. doi: 10.1089/vbz.2014.1657

Himsworth, C. G., Jardine, C. M., Parsons, K. L., Feng, A. Y., and Patrick, D. M. (2014a). The characteristics of wild rat (*Rattus* spp.) populations from an inner-city neighborhood with a focus on factors critical to the understanding of rat-associated zoonoses. *PLoS ONE* 9:e91654. doi: 10.1371/journal.pone.0091654

Himsworth, C. G., Parsons, K. L., Feng, A. Y., Kerr, T., Jardine, C. M., and Patrick, D. M. (2014b). A mixed methods approach to exploring the relationship between Norway rat (*Rattus norvegicus*) abundance and features of the urban environment in an inner-city neighborhood of Vancouver, Canada. *PLoS ONE* 9:e97776. doi: 10.1371/journal.pone.0097776

Himsworth, C. G., Parsons, K. L., Jardine, C., and Patrick, D. M. (2013). Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne Zoonotic Dis.* 13, 349–359. doi: 10.1089/vbz.2012.1195

Hopkins, G. H. E. (1949). *Report on Rats, Fleas and Plague in Uganda. East African Standard Ltd.* For the Government Printer of Uganda. 52.

Hsieh, J. W., Tung, K. C., Chen, W. C., Lin, J. W., Chien, L. J., Hsu, Y. M., et al. (2010). Epidemiology of *Bartonella* infection in rodents and shrews in Taiwan. *Zoonoses Public Health* 57, 439–446. doi: 10.1111/j.1863-2378.2009.01234.x

Inoue, K., Maruyama, S., Kabeya, H., Yamada, N., Ohashi, N., Sato, Y., et al. (2008). Prevalence and genetic diversity of *Bartonella* species isolated from wild rodents in Japan. *Appl. Environ. Microbiol.* 74, 5086–5092. doi: 10.1128/AEM.00071-08

Jiyipong, T., Jittapalapong, S., Morand, S., Raoult, D., and Rolain, J. M. (2012). Prevalence and genetic diversity of *Bartonella* spp. *i*n small mammals from Southeastern Asia. *Appl. Environ. Microbiol.* 78, 8463–8466. doi: 10.1128/AEM.02008-12

Kamani, J., Morick, D., Mumcuoglu, K. Y., and Harrus, S. (2013). Prevalence and diversity of *Bartonella* species in commensal rodents and ectoparasites from Nigeria, West Africa. *PLoS Negl. Trop. Dis.* 7:e2246. doi: 10.1371/journal.pntd.0002246

Kandelaki, G., Malania, L., Bai, Y., Chakvetadze, N., Katsitadze, G., Imnadze, P., et al. (2016). Human Lymphadenopathy caused by ratborne Bartonella, Tbilisi, Georgia. *Emerg. Infect. Dis.* 22, 544–546. doi: 10.3201/eid2203.151823

Khlyap, L., Glass, G., and Kosoy, M. (2012). "Rodents in urban ecosystems of Russia and the USA," in *Rodents: Habitat, Pathology and Environmental Impact.* eds A. Triunveri and D. Scalise (Nova Scientific Publishers), 1–22.

Khlyap, L. A., Kosoy, M., Popov, V. P., Cosson, J. F., and Morand, S. (2016). Rats of the genus Rattus as hosts for natural focal infectious agents. *Med. Parazitol (Mosk).* 47–52.

Kim, K. S., Inoue, K., Kabeya, H., Sato, S., Takada, T., and Pangjai, D., et al (2016). Prevalence and diversity of *Bartonella* species in wild small mammals in Asia. *J. Wildl. Dis.* 52, 10–21. doi: 10.7589/2015-01-015

Klangthong, K., Promsthaporn, S., Leepitakrat, S., Schuster, A. L., McCardle, P. W., Kosoy, M., et al. (2015). The distribution and diversity of *Bartonella* species in rodents and their ectoparasites across Thailand. *PLoS ONE* 10:e0140856. doi: 10.1371/journal.pone.0140856

Kosoy, M., Bai, Y., Sheff, K., Morway, C., Baggett, H., Maloney, S. A., et al. (2010). Identification of Bartonella infections in febrile human patients from Thailand and their potential animal reservoirs. *Am. J. Trop. Med. Hyg.* 82, 1140–1145. doi: 10.4269/ajtmh.2010.09-0778

Kosoy, M., Hayman, D. T., and Chan, K. S. (2012). Bartonella bacteria in nature: where does population variability end and a species start? *Infect. Genet. Evol.* 12, 894–904. doi: 10.1016/j.meegid.2012.03.005

Kosoy, M., Khlyap, L., Cosson, J. F., and Morand, S. (2015). Aboriginal and invasive rats of genus *Rattus* as hosts of infectious agents. *Vector Borne Zoonotic Dis.* 15, 3–12. doi: 10.1089/vbz.2014.1629

Kosoy, M., McKee, C., Albayrak, L., and Fofanov, Y. (2018). Genotyping of *Bartonella* bacteria and their animal hosts: current status and perspectives. *Parasitology* 145, 543–562. doi: 10.1017/S0031182017001263

Kosoy, M. Y., Regnery, R. L., Tzianabos, T., Marston, E. L., Jones, D. C., Green, D., et al. (1997). Distribution, diversity, and host specificity of *Bartonella* in rodents from the southeastern United States. *Am. J. Trop. Med. Hyg.* 57, 578–588. doi: 10.4269/ajtmh.1997.57.578

Krasnov, B. R., Khokhlova, I. S., Fielden, L. J., and Burdelova, N. V. (2001). Effect of air temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: Pulicidae). *J. Med. Entomol.* 38, 629–637. doi: 10.1603/0022-2585-38.5.629

Lack, J. B., Greene, D. U., Conroy, C. J., Hamilton, M. J., Braun, J. K., Mares, M. A., et al. (2012). Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. *Mol. Ecol.* 21, 3545–3561. doi: 10.1111/j.1365-294X.2012.05620.x

Lack, J. B., Hamilton, J., Braun, J. K., Mares, M. A., and Van Den Bussche, R. A. (2013). Comparative phylogeography of invasive *Rattus rattus* and *Rattus norvegicus* in the U.S. reveals distinct colonization histories and dispersal. *Biol. Invasions* 15, 1067–1087. doi: 10.1007/s10530-012-0351-5

Laudisoit, A., Falay, D., Amundala, N., Akaibe, D., de Bellocq, J. G., Van Houtte, N., et al. (2014). High prevalence of *Rickettsia typhi* and *Bartonella* species in rats and fleas, Kisangani, Democratic Republic of the Congo. *Am. J. Trop. Med. Hyg.* 90, 463–468. doi: 10.4269/ajtmh.13-0216

Lin, J. W., Chen, C. Y., Chen, W. C., Chomel, B. B., and Chang, C.C. (2008). Isolation of Bartonella species from rodents in Taiwan including a strain closely related to *'Bartonella rochalimae'* from *Rattus norvegicus*. *J. Med. Microbiol.* 57(Pt 12), 1496–1501. doi: 10.1099/jmm.0.2008/004671-0

Loan, H. K., Cuong, N. V., Takhampunya, R., Klangthong, K., Osikowicz, L., Kiet, B. T., et al. (2015). *Bartonella* species and trombiculid mites of rats from the Mekong Delta of Vietnam. *Vector Borne Zoonotic Dis.* 15, 40–47. doi: 10.1089/vbz.2014.1604

Lund, M. (2015). "Commensal rodents," in *Rodent pests and their control. CAB International*, *2nd Edn,* eds. A. P. Buckle and R. H. Smith, (Wallingford Oxon: University of Huddersfield), 23–43.

Márquez, F. J., Rodríguez-Liébana, J. J., Pachón-Ibáñez, M. E., Docobo-Pérez, F., Hidalgo-Fontiveros, A., Bernabeu-Wittel, M., et al. (2008). Molecular screening of *Bartonella* species in rodents from south western Spain. *Vector Borne Zoonotic Dis.* 8, 695–700. doi: 10.1089/vbz.2007.0257

Martin-Alonso, A., Houemenou, G., Abreu-Yanes, E., Valladares, B., Feliu, C., and Foronda, P. (2016). Bartonella spp. in Small Mammals, Benin. *Vector Borne Zoonotic Dis*. 16, 229–237. doi: 10.1089/vbz.2015.1838

Martin-Alonso, A., Soto, M., Foronda, P., Aguilar, E., Bonnet, G., Pacheco, R., et al. (2014). *Bartonella* spp. and *Yersinia pestis* reservoirs, Cusco, Peru. *Emerg. Infect. Dis*. 20, 1069–1070. doi: 10.3201/eid2006.131194

McFarlane, R., Sleigh, A., and McMichael, T. (2012). Synanthropy of wild mammals as a determinant of emerging infectious diseases in the Asian-Australasian region. *Ecohealth* 9, 24–35. doi: 10.1007/s10393-012-0763-9

McKee, C. D., Osikowicz, L. M., Schwedhelm, T. R., Maes, S. E., Enscore, R. E., Gage, K. L., et al. (2018). Acquisition of *Bartonella elizabethae* by experimentally exposed Oriental rat fleas (*Xenopsylla cheopis*; Siphonaptera, Pulicidae) and excretion of Bartonella DNA in flea feces. *J. Med. Entomol.* 55, 1292–1298. doi: 10.1093/jme/tjy085

McVea, D. A., Himsworth, C. G., Patrick, D. M., Lindsay, L. R., Kosoy, M., and Kerr, T. (2018). Exposure to rats and rat-associated *Leptospira* and *Bartonella* species among people who use drugs in an impoverished, inner-city neighborhood of Vancouver, Canada. *Vector Borne Zoonotic Dis.* 18, 82–88. doi: 10.1089/vbz.2017.2179

Meheretu, Y., Leirs, H., Welegerima, K., Breno, M., Tomas, Z., Kidane, D., et al. (2013). *Bartonella* prevalence and genetic diversity in small mammals from Ethiopia. *Vector Borne Zoonotic Dis.* 13, 164–175. doi: 10.1089/vbz.2012.1004

Morick, D., Baneth, G., Avidor, B., Kosoy, M. Y., Mumcuoglu, K. Y., and Mintz, D. (2009). Detection of *Bartonella* spp. in wild rodents in Israel using HRM real-time PCR. *Vet. Microbiol*. 139, 293–297. doi: 10.1016/j.vetmic.2009.06.019

Musser, G. G., and Carlton, M. D. (2005). "Superfamily Muroidea," in *Mammal Species of the World, 3rd Edn,* eds D. E. Wilson and D. M. Reeder (The Johns Hopkins University Press), 894–1531.

Myint, K. S., Gibbons, R. V., Iverson, J., Shrestha, S. K., Pavlin, J. A., Mongkolsirichaikul, D., et al. (2011). Serological response to *Bartonella* species in febrile patients from Nepal. *Trans. R. Soc. Trop. Med. Hyg.* 105, 740–742. doi: 10.1016/j.trstmh.2011.08.002

Neves, E. S., Mendenhall, I. H., Borthwick, S. A., Su, Y. C. F., and Smith, G. J. D. (2018). Detection and genetic characterization of diverse *Bartonella* genotypes in the small mammals of Singapore. *Zoonoses Public Health* 65, e207–e215. doi: 10.1111/zph.12430

Papkou, A., Gokhale, C. S., Traulsen, A., and Schulenburg, H. (2016). Host-parasite coevolution: why changing population size matters. *Zoology* 119, 330–338. doi: 10.1016/j.zool.2016.02.001

Peterson, A. C., Ghersi, B. M., Alda, F., Firth, C., Frye, M. J., Bai, Y., et al. (2017). Rodent-borne *Bartonella* infection varies according to host species within and among cities. *Ecohealth* 14, 771–782. doi: 10.1007/s10393-017-1291-4

Pretorius, A. M., Beati, L., and Birtles, R. J. (2004). Diversity of *Bartonellae* associated with small mammals inhabiting Free State province, South Africa. *Int. J. Syst. Evol. Microbiol.* 54, 1959–1967. doi: 10.1099/ijs.0.03033-0

Psaroulaki, A., Antoniou, M., Toumazos, P., Mazeris, A., Ioannou, I., Chochlakis, D., et al. (2010). Rats as indicators of the presence and dispersal of six zoonotic microbial agents in Cyprus, an island ecosystem: a seroepidemiological study. *Trans. R. Soc. Trop. Med. Hyg.* 104, 733–739. doi: 10.1016/j.trstmh.2010.08.005

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *Am. Naturalist* 132, 652–661. doi: 10.1086/284880

Rothenburger, J. L., Himsworth, C. G., Nemeth, N. M., Pearl, D. L., and Jardine, C. M. (2018). Beyond abundance: how microenvironmental features and weather influence *Bartonella tribocorum* infection in wild Norway rats (*Rattus norvegicus*). *Zoonoses Public Health* 65, 339–351. doi: 10.1111/zph.12440

Saisongkorh, W., Wootta, W., Sawanpanyalert, P., Raoult, D., and Rolain, J. M. (2009). "*Candidatus* Bartonella thailandensis": a new genotype of *Bartonella* identified from rodents. *Vet. Microbiol.* 139, 197–201. doi: 10.1016/j.vetmic.2009.05.011

Schülein, R., Seubert, A., Gille, C., Lanz, C., Hansmann, Y., Piémont, Y., et al. (2001). Invasion and persistent intracellular colonization of erythrocytes. A unique parasitic strategy of the emerging pathogen *Bartonella*. *J. Exp. Med.* 193, 1077–1086.

Segers, F. H., Kešnerová, L., Kosoy, M., and Engel, P. (2017). Genomic changes associated with the evolutionary transition of an insect gut symbiont into a blood-borne pathogen. *ISME J.* 11, 1232–1244. doi: 10.1038/ismej.2016.201

Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., and Hope, D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol. Evol.* 21, 186–191. doi: 10.1016/j.tree.2005.11.019

Tay, S. T., Mokhtar, A. S., Zain, S. N., and Low, K. C. (2014). Isolation and molecular identification of Bartonella from wild rats (Rattus species) in Malaysia. *Am. J. Trop. Med. Hyg.* 90, 1039–1042. doi: 10.4269/ajtmh.13-0273

Trataris, A. N., Rossouw, J., Arntzen, L., Karstaedt, A., and Frean, J. (2012). *Bartonella* spp. in human and animal populations in Gauteng, South Africa, from 2007 to 2009. *Onderstepoort J. Vet. Res.* 79, 452–460. doi: 10.4102/ojvr.v79i2.452

Tsai, Y. L., Chuang, S. T., Chang, C. C., Kass, P. H., and Chomel, B. B. (2010). *Bartonella* species in small mammals and their ectoparasites in Taiwan. *Am. J. Trop. Med. Hyg.* 83, 917–923. doi: 10.4269/ajtmh.2010.10-0083

Vayssier-Taussat, M., Moutailler, S., Féménia, F., Raymond, P., Croce, O., La Scola, B., et al. (2016). Identification of novel zoonotic activity of *Bartonella* spp., France. *Emerg. Infect. Dis.* 22, 457–462. doi: 10.3201/eid2203.150269

Vicente, S., and Gómez López, M. (2012). Presence of *Stenoponia tripectinata* (Tiraboschi, 1902) (Siphonaptera, Ctenophtalmidae) in murine (Rodentia) from the Canary Islands. *Acta Parasitol.* 57, 190–193. doi: 10.2478/s11686-012-0024-7

Winoto, I. L., Goethert, H., Ibrahim, I. N., Yuniherlina, I., and Stoops, C., Susanti, I., et al. (2005). *Bartonella* species in rodents and shrews in the greater Jakarta area. *Southeast Asian J. Trop. Med. Public Health* 36, 1523–1529.

Ye, X., Li, G. W., Yao, M. L., Luo, W., and Su, L. Q. (2009). Study on the prevalence and genotypes of *Bartonella* species in rodent hosts from Fujian coastal regions. *Zhonghua Liu Xing Bing Xue Za Zhi*. 30, 989–992.

Ying, B., Kosoy, M. Y., Maupin, G. O., Tsuchiya, K. R., and Gage, K. L. (2002). Genetic and ecologic characteristics of *Bartonella* communities in rodents in southern China. *Am. J. Trop. Med. Hyg.* 66, 622–627. doi: 10.4269/ajtmh.2002.66.622

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REVIEW
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Parameters That Affect Fear Responses in Rodents and How to Use Them for Management

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The strong innate fear response shown by laboratory rodents to predator cues could provide powerful and innovative tools for pest management. Predator cues are routinely used to induce fear and anxiety in laboratory rodents for pharmacological studies. However, research on the fear response induced by predator cues in different species of rodents in the wild has been inconclusive with results often contradictory to laboratory experiments. Potential explanations for this inconsistency include the prey's: (i) physiological state; (ii) parasite load; (iii) differential intensity of perceived threats; (iv) fear learning and habituation; and (v) information gathering. In this review, we first explore current knowledge on the sensory mechanisms and capabilities of rodents, followed by the discussion of each of these explanations within the context of their implications for the use of antipredator response as a pest rodent management tool. Finally, we make recommendations on potential solutions and strategies to resolve issues in rodent management related to these hypotheses.

Keywords: non-consumptive effects, anti-predator response, fear, predator cues, learning, fear conditioning, pest rodents

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INTRODUCTION

Worldwide, rodents are prolific and pervasive pests, destroying crops, spreading disease, and causing enormous damage to infrastructure (Mills, 1999; Meerburg et al., 2009a,b). From a conservation perspective, introduced pest rodents have been linked to the demise of many native species around the world, particularly on islands (Atkinson, 1973, 1985; Capizzi et al., 2014). In several developing countries, rodents are considered the main cause of agricultural losses (Makundi et al., 1999). It is estimated that globally 77 million tons of food are lost annually due to rodent pests (John, 2014). In Asia alone, the annual consumption of food crops by rodents could feed 200 million people (Singleton, 2003). Due to rodents, rice harvest in Indonesia is reduced by 15% annually (Geddes, 1992). Tanzania loses US$45 million every year in reduced maize yield (Leirs, 2003) and in some areas of South America, rodent related damage to crops can amount to up to 90% of the total annual production (Rodriguez, 1993). A recent review on the impact of pest rodents in Africa, found that losses fall between 20 and 50% (Swanepoel et al., 2017). Overall, damage to pre and post-harvest crops affects approximately 280 million undernourished people worldwide (Stenseth et al., 2003; Meerburg et al., 2009b). Yet, the number of species of rodents that are consider pests, represents <10% of all the rodents species currently known (Singleton et al., 2007).

In most urban areas around the world, commensal rodents are common and live alongside humans in houses, buildings and other infrastructure such as sewers (Tobin and Fall, 2006). At high densities, rodents contaminate food, damage infrastructure, increase risk of fire by gnawing on electrical wiring and pose a risk to public health as diseases carriers (Tobin and Fall, 2006; Meerburg et al., 2009a; Almeida et al., 2013; Buckle and Smith, 2015). Wherever humans thrive, pest rodents do as well (Barnett, 2001; Lund, 2015).

The most common approach to rodent management follows the well-known framework of Integrated Pest Management (IPM; Singleton et al., 1999; Tobin and Fall, 2006). The IPM model is the combination of all available pest control methods with preventative measures to reduce subsequent pest population increases, while ensuring that these techniques are economically justified and do not pose a risk to human health and the environment (Food Agriculture Organization of the United Nations, 2018). IPM commonly includes monitoring, sanitation, physical interventions (e.g., exclusion, traps, deterrents), and ultimately rodenticides (Kaukeinen, 1994; Singleton et al., 1999; Bennett et al., 2003).

Physical interventions and the use of rodenticides can be particularly difficult to implement, due to difficulties intrinsic to rodent physiology and behavioral adaptations. Most pest rodent species show high levels of neophobia (Barnett, 1958; Barnett and Cowan, 1976; Meehan, 1984), both towards novel objects and tastes (Domjan, 1975; Rusiniak et al., 1979), which results in high levels of "trap-shyness" (Chitty, 1954; Taylor et al., 1974), and low bait acceptance (Brunton et al., 1993; Inglis et al., 1996). Rodents also learn from the experience of conspecifics (Lore et al., 1971); if conspecifics emit signals of distress—e.g., getting caught in a trap—they are less likely to approach the same area later on (Brudzynski and Chiu, 1995; Brechbühl et al., 2013; Haapakoski et al., 2018). Furthermore, the widespread use of rodenticides have induced the development of resistance in rodent populations to first and second-generation anticoagulant poisons (e.g., warfarin, bromadiolone, difenacoum, chlorophacinone; Thijssen, 1995; Pelz et al., 2005; Pelz, 2007; Rost et al., 2009; Buckle, 2013; Meerburg et al., 2014). Concurrently, the widespread use of these poisons can have considerable negative impacts on non-target wildlife (Howald et al., 1999; Eason et al., 2002; Lambert et al., 2007; Walker et al., 2008; Albert et al., 2010; Dowding et al., 2010; Lima and Salmon, 2010; Thomas et al., 2011; Gabriel et al., 2012; Elliott et al., 2014; Coeurdassier et al., 2018; Lohr and Davis, 2018; Rattner and Mastrota, 2018). The development of alternative and innovative ways of managing rodent pests is therefore of high importance.

History can provide inspiration for new and innovative ways to manage rodent pests. One of the oldest methods of controlling rodents is the use of cats. Cats started their domestication serendipitously as commensal relationships with humans, feeding upon the rodents that infested the stored grain of farmers (Clutton-Brock, 1999). Yet, the effect cats have on rodent populations may be more complex that just population reduction. The effect predators have on prey is not only defined by lethal interactions (Taylor, 1984), but also non-lethal effects. For example, in agricultural settings, the protection guard dogs provide to livestock is through deterrence instead of actual attacks and killing of predators (Hansen and Bakken, 1999; van Eeden et al., 2017). Hence, analogous rodent-deterrence strategies may be of value.

Risk of predation is ubiquitous to almost all taxa, and as such all species show some level of antipredator defense (Freeland, 1991; Caro, 2005). These defenses can be constitutive (e.g., spines in porcupines, thorns in plants; Fraenkel, 1959; Tollrian and Harvell, 1999) or inducible (e.g., morphology in tadpoles, coloration in some crustaceans, and behavioral modifications; Kerfoot and Sih, 1987; Harvell and Tollrian, 1999; Tollrian and Harvell, 1999; Creel et al., 2007; Schoeppner and Relyea, 2009). Constitutive defenses are favored when risk is constantly high and/or defenses are cheap, while inducible defenses are favored when risk is variable and defenses are costly (Tollrian and Harvell, 1999). Inducible defenses allow control on defense expression according to risk level, thus reducing the costs associated with it (Tollrian and Harvell, 1999). Inducible antipredator responses are expected to evolve only if the costs associated with them are offset by their effectiveness in reducing the rate of direct predation.

The antipredator responses and their associated costs drive an evolutionary arms race between predator and prey, and constitute what is known as the "ecology of fear" (Brown et al., 1999), "degree of fear" (Stankowich and Blumstein, 2005), or "cost of fear" (Martin, 2011). The costs of anti-predator responses can include reduced survival (Dudeck et al., 2018; MacLeod et al., 2018); growth (Pangle et al., 2007); fecundity (Ruxton and Lima, 1997; Naidenko et al., 2003; Voznessenskaya et al., 2003; Fuelling and Halle, 2004; Creel et al., 2007) and reproduction (Zanette et al., 2011; Bonnington et al., 2013; Dudeck et al., 2018). More recently, limited evidence have shown that predation risk can drive an increase in current reproductive investment with associated costs to future reproduction (Duffield et al., 2017; Haapakoski et al., 2018). In some extreme cases, fear can induce the development of chronic stress conditions similar to Post-Traumatic Stress Disorders (PTSD) not only in humans, but also in rodents, primates and rabbits (Clinchy et al., 2013). These costs are sometimes more important to the prey population than the lethal effects themselves (Brown and Alkon, 1990; Schmitz et al., 1997; Matassa and Trussell, 2011).

It is theoretically possible to use these non-consumptive costs as a way managing pest rodent populations (Singleton et al., 1999). Pest controllers could manipulate how commensal rodents perceive predation risk to deter them from areas of interest (e.g., crops, food storage facilities). The Landscape of Fear (LOF) framework is a theoretical tool that allows measurement of the way an animal perceives its environment, based on a trade-off between resources and safety, linked to specific areas of available habitat (Laundré et al., 2001), and thus is a spatial representation of the use of habitat by a prey species. This framework has been supported across a wide range of systems (Bleicher, 2017). The LOF is considered the basis in which the use of perceived predation risk as a management tool against pest rodents can be built upon (Krijger et al., 2017).

A major obstacle in the development of a fear-inducing rodent management technique is the variation in anti-predator

responses displayed by wild rats and mice in comparison with their laboratory counterparts. Laboratory rodents respond to a myriad of different predator cues (Vernet-Maury et al., 1984; Blanchard R.J et al., 1991; Dielenberg and McGregor, 2001; Mongeau et al., 2003; Litvin et al., 2007; Kendig et al., 2011; Bowen et al., 2013; Wallace et al., 2013; Yilmaz and Meister, 2013; Ayral et al., 2015), and also demonstrate anxiety-like behaviors, often utilized to model the human condition (Apfelbach et al., 2005; Staples et al., 2008). However, evidence of wild rodents responding to predator cues is inconclusive. Studies with captive wild-type brown rats (*Rattus norvegicus*) (Berdoy and Macdonald, 1991; Macdonald et al., 1999) and wild caught black rats (*Rattus rattus*) (Burwash et al., 1998) showed antipredator responses consistent to those of their laboratory counterparts. Some field studies demonstrated that wild black rats show aversion to fox and cat feces (Banks, 1998), as well as changes in habitat use in the presence of dogs and cats (Mahlaba et al., 2017). In contrast, other studies have shown either no effect of ferret, cat or mongoose odors in black rat visitation (Garvey et al., 2017) and foraging (Bramley and Waas, 2001); and in more extreme cases, black rats were attracted and visitation increased in response to cat body odor (Carthey and Banks, 2016).

Several explanations for the variability in rodent responses to predator cues have been put forward, namely the prey's: (i) physiological state (Abrams, 1994); (ii) parasite loads (Macdonald et al., 1999); (iii) differential intensity of perceived threats (Kavaliers and Choleris, 2001); (iv) fear learning and habituation (Schulte, 2016); and (v) information gathering (Parsons et al., 2017). In this review, we first explore the current knowledge on the sensory mechanisms and capabilities of rodents, followed by the discussion of each of these explanations within the context of their implications for the use of antipredator response as a pest rodent management tool.

SENSORY CAPABILITIES OF RODENTS

Before an animal responds to a predator, it needs to be able to detect its presence. Rodents can detect and respond to visual (Wallace et al., 2013; Yilmaz and Meister, 2013); auditory (Blanchard R.J et al., 1991; Mongeau et al., 2003; Litvin et al., 2007), and olfactory (Vernet-Maury et al., 1984; Dielenberg and McGregor, 2001; Kendig et al., 2011; Bowen et al., 2013; Ayral et al., 2015) predator cues, highlighting that their sensory capabilities are highly tuned to predator detection.

Sight

Laboratory rodents are commonly used as models to study the mammalian visual system (Hughes, 1979; Remtulla and Hallett, 1985; Legg and Lambert, 1990; Berardi and Maffei, 1999; Zoccolan et al., 2009). As rodents are predominantly prey species, they have laterally facing eyes that allow for a panoramic field of view that extends forwards and also covers the back of the animals head, enhancing potential threat detection (Hughes, 1979; Remtulla and Hallett, 1985). Through eye movement alone, rodents are capable of overlapping the fields of view of both eyes to obtain binocular vision, at the loss of a complete panoramic field of view (Wallace et al., 2013). Binocular vision is important for judging distance (Russell, 1932; Legg and Lambert, 1990) and visual acuity (Hughes, 1979; Remtulla and Hallett, 1985; Zoccolan et al., 2009), while a panoramic monocular vision allows for wide surveillance (Hughes, 1979; Remtulla and Hallett, 1985).

Due to the reliability of visual cues—i.e., seeing a predator is a perfect clue that there is a high risk of predation—most species, including rodents, respond to oversimplified representations of predators (Kavaliers and Choleris, 2001). Some of these representations can encompass only size (Hanson and Coss, 1997; Mathis and Vincent, 2000), shape (Coss and Ramakrishnan, 2000; Emile and Barros, 2009), coloration (Kelley and Magurran, 2003), movement (Yilmaz and Meister, 2013), or frontally positioned eyes (Topál and Csányi, 1994). Rodents in particular, are highly sensitive to movement (Wallace et al., 2013; Yilmaz and Meister, 2013). When exposed to an oversimplified looming stimulus simulating a raptor, laboratory rats maintain overhead binocular vision (Wallace et al., 2013), thus, enabling them to judge the raptor's elevation.

Hearing

Mice and rats are particularly attuned to detect high frequency sounds, often higher than humans have the ability to detect (Heffner and Heffner, 2007, 2008). Accordingly, these rodents commonly produce high-frequency sounds that have been linked to conspecific communication (Portfors, 2007). In laboratory rodents, con-specific high-frequency alarm calls are used to communicate threats (Brudzynski and Chiu, 1995). However, rodents are also capable of detecting lower frequency sounds (Heffner and Heffner, 2007, 2008). Studies in voles and gray squirrels have shown that playback of raptor calls can incite antipredator responses (Bohls and Koehnle, 2017; Lyly et al., 2018).

Smell

In most mammals, olfaction is the most developed sensory system (Eisenberg and Kleiman, 1972), and rodents are no exception (Vernet-Maury et al., 1984; Dielenberg and McGregor, 2001; Kendig et al., 2011; Bowen et al., 2013; Ayral et al., 2015). In contrast with the singularity of visual (i.e., retina) and auditory (i.e., ear) sensing, smells are processed by multiple distinct olfactory systems, involving distinct receptor organs and central neuronal processes involved in detection (Chamero et al., 2012; Ma, 2012). The main olfactory system processes scents and flavors, while the accessory olfactory system processes con-specific and heterospecific chemical cues (pheromones and kairomones) (Ma, 2012).

Predator recognition by olfactory cues does not require cortical information processing (Canteras et al., 2015). Discrimination of different odorants is achieved by the narrow sensitivity to chemical cues from each receptor type in the accessory olfactory system (AOS; Mucignat-Caretta, 2010; Ma, 2012; Canteras et al., 2015; Tromelin, 2016). In laboratory rodents, different predator odors activate receptors from distinct olfactory subsystems (Canteras et al., 2015). Carnivore urine activates TAAR4 neurons in the main olfactory epithelium (Ferrero et al., 2011; Dewan et al., 2013), cat fur activates vomeronasal organ receptors (McGregor et al., 2004), and stoat

anal gland smells activate receptors in the Grueneberg ganglion (Pérez-Gómez et al., 2015). Therefore, activation of specific receptors allows for the recognition of specific predators. In the following section we will describe how after detection and recognition is achieved an appropriate response is induced. Interestingly, rodents are also capable of utilize olfactory signaling from conspecific in order to asses predation risk (Abel, 1991; Kikusui et al., 2001; Haapakoski et al., 2018). And in some cases these pheromones activate similar receptors as predator odors (Brechbühl et al., 2008, 2013).

Sensory Coordination and Response

On predator detection all sensory signals, regardless of their origin (i.e., visual, auditory or chemical), converge in the amygdala (Krettek and Price, 1977; Campeau and Davis, 1995; Chamero et al., 2012; Ma, 2012; Pérez-Gómez et al., 2015). Amygdala signaling initiates sensory and motor response coordination to the predatory threat (Miller and Vogt, 1984) provoking appropriate behavioral and physiological responses (Campeau and Davis, 1995), such as freezing or evasive behaviors. Thus, differences in rodents' antipredator responses are expected to arise because of differences in detection, recognition, or response to predator cues, mediated through complex but converging neural signaling. Modulation of this signaling pathway can occur at every stage based on the individual's internal state, prior experience, or the context, leading to observable variability in antipredator responses.

VARIABILITY IN RODENT RESPONSES TO PREDATOR CUES

Prey's Physiological State

The risk of predation is ubiquitous to all animals (Freeland, 1991; Caro, 2005), however so is the need to forage and acquire resources (Charnov, 1979; Stephens and Krebs, 1986). Yet, laboratory rodents are commonly kept in standard captive husbandry conditions with *ad libitum* access to food and water, and controlled environmental variables such as temperature, humidity and photoperiod (Allmann-lselin, 2000; Hedrich and Bullock, 2004; National Research Council, 2010). In comparison, wild rodents must cope with a variety of environmental factors, while balancing the risk of predation and resource acquisition to optimize their fitness (Abrams, 1994).

Starvation alters the antipredator response in both laboratory (Shoham et al., 2000; Verma et al., 2016) and wild rodents. Starved captive wild-caught Anderson's gerbils (*Gerbillus andersoni allenbyi*) increase foraging despite predation risk (Berger-Tal and Kotler, 2010; Berger-Tal et al., 2010). Free-ranging deer mice (*Peromyscus maniculatus*) (Morris, 1997; Davidson and Morris, 2001) and house mice (*Mus musculus domesticus*) (Ylönen et al., 2002) reduce their foraging at the cost of increased predation risk, when the population density is high driving higher intraspecific competition and thus lower internal energetic state (Bedoya-Pérez et al., 2013).

Relative resource quality also alters the intensity of anti-predator responses (Thorson et al., 1998). Fox squirrels (*Sciurus niger*) (Brown and Morgan, 1995), Namaqua mouse (*Micaelamys namaquensis*) (Abu Baker and Brown, 2012), Anderson's gerbils (*G. andersoni allenbyi*), the greater Egyptian gerbil (*G. pyramidurn*) (Garb et al., 2000), Merriam's kangaroo rats (*Dipodomys merriami*), and pocket mice (*Chaetodipus* spp.) (Leaver and Daly, 2003) forage more intensely on highly nutritious food regardless of predation risk. Similarly, fox squirrels (*S. niger*), gray squirrels (*S. carolinensis*) (Schmidt et al., 1998), as well as the African unstriped ground squirrel (*Xerus rutilus)* (Fanson et al., 2010), reduce foraging on poor quality food and become more sensitive to predation risk (Bedoya-Pérez et al., 2013).

Other physiological, developmental, and reproductive factors can alter an animal's anti-predator response. For example, immunochallenged white-footed mice (*Peromyscus leucopus)* forage more and are less selective in their habitat use despite the risk of predation (Schwanz et al., 2011, 2012), and Anderson's gerbils (*G. andersoni allenbyi*) and the greater Egyptian gerbil (*G. pyramidurn*) increase foraging efforts during the reproductive season (Kotler et al., 2004). More relevant to rodent management, we know that pest rodents infected with *Toxoplasma gondii* showed a reduced aversion to predators (Berdoy et al., 1995a, 2000; Webster, 2007), we will this discuss this particular case in more detail in the following section.

To exploit anti-predator responses as a pest management tool, it is essential to recognize that wild rodents are not well-fed, homeostatic animals like their laboratory counterparts. If resources are low, and pest rodents are at risk of starvation, these animals are expected to show less-pronounced anti-predator behaviors than those shown by laboratory animals (Abrams, 1994).

Parasite Loads the Case of *Toxoplasma gondii*

Parasite loads can alter an animals perceived predation risk either by altering their internal physiological state (Schwanz et al., 2011, 2012), or through more complex mechanisms involving modulation of neuronal pathways (Berdoy et al., 1995a; Raveh et al., 2011). The complete disruption of anti-predator response in rodents infected by *Toxoplasma gondii* has received wide attention in recent years (Webster, 2007).

Toxoplasma gondii is a parasitic protozoan capable of infecting all mammals, including humans (Hutchison et al., 1969). Domestic cats and other felines are the final host of the parasite, and are the only animals known to shed the parasite's oocyst in their faces (Hutchison et al., 1969). Transmission of the parasite can occur from cat to cat, but more commonly involves an intermediate host, such as a rodent. Here the parasite multiplies and forms cysts in almost every organ, particularly in the brain (Hutchison et al., 1969; Berdoy et al., 1995b). When a cat predates upon an infected rodent, the parasite passes to the final host where sexual reproduction occurs (Berdoy et al., 1995b).

One of the first studies demonstrating a connection between *T. gondii* infections and changes in behavior showed that laboratory rats and mice were cognitively impaired by the parasite (Piekarski et al., 1978). Subsequent studies have showed that *Toxoplasma*-infected rats and mice display deficits in

both learning capacities (Witting, 1979) including novel object recognition (Hutchison et al., 1980; Webster et al., 1994). It was suggested that the reduction in neophobia could cause rats to be more susceptible to predation (Webster et al., 1994). It was later demonstrated that *Toxoplasma*-infected rats are more likely to approach areas with signs of cats, although retaining certain level of innate aversion (Berdoy et al., 1995a). These findings were confirmed by the demonstration that, compared to non-infected rats, *Toxoplasma*-infected rats visited areas treated with cat urine more often that control areas or areas treated with rabbit urine (Berdoy et al., 2000; Webster, 2007).

Toxoplasma gondii likely does not alter the sensory detection of the threat (Vyas et al., 2007a), but instead modifies the learning processes specifically related to cat odor (Vyas et al., 2007a,b). Neurobiologically, *T. gondii* causes epigenetic changes in the DNA methylation in the medial amygdala causing greater expression of arginine vasopressin promoter (Hari Dass and Vyas, 2014). The infection also causes retraction of dendritic processes in basolateral amygdala neurons, reducing the amount of circulating corticosterone (Mitra et al., 2012). A reduction in corticosterone disrupts both the fight or flight response as well-fear memory consolidation (Stephens and Wand, 2012). Behaviorally, infected laboratory rats show reduced anxiety-like behaviors in exploration-based testing, but not during social interaction testing (Gonzalez et al., 2007). This may be because *T. gondii* favors vertical transmission (i.e., cat predation) over horizontal transmission (i.e., infected parents to offspring; Vyas and Sapolsky, 2010). However, research shows that the mechanisms involved in behavioral alterations in male rodents increases sexual arousal (House et al., 2011) and attractiveness (Dass et al., 2011), potentially allowing the parasite to be transmitted sexually and congenitally (Beverley, 1959, 1976; Dass et al., 2011).

In terms of rodent management, undoubtedly *T. gondii* can have profound consequences for the use of anti-predator responses as a tool. However, these consequences are dependent on the prevalence of the infection in the targeted pest rodent population. It is unlikely that this protozoan is the cause of the variation in response between laboratory and field studies, when the prevalence in the field is appreciatively low (Carthey and Banks, 2016). In cases where the prevalence may be high, the disruption of antipredator behavior by *T. gondii* could be used synergistically with predator cues. That is, other management tools (e.g., lethal and non-lethal traps) could be deployed at the same locations as predator cues, thus infected rodents could be removed, while uninfected may be repelled. This method may reduce the prevalence of *T. gondii*, not only in the rodent population, but at the community level. Reductions in the number of infected carriers would benefit non-specific host species that may be vulnerable to the infection, such as humans (Tenter et al., 2000; Dubey and Jones, 2008) and several endangered species that are more susceptible to develop negative symptoms from the infection [e.g., Eastern quoll, *Dasyurus viverrinus* (Fancourt and Jackson, 2014; Fancourt et al., 2014), Tamar wallabies, *Macropus eugenii,* and Bennett's wallabies, *Macropus rufogriseus* (Dubey and Crutchley, 2008)].

Due to the extensive and ever-growing body of knowledge on the mechanisms and potential consequences of *T. gondii,* we have focused on this particular parasite. However, little is known about the potential effects that other parasites may have in the anti-predator behavior of pest rodents. Some evidence suggests that some infections and diseases may have overarching population level consequences such as increase in reproduction (Duffield et al., 2017).

Differential Intensity of Perceived Threats

There are considerable differences between laboratory and field studies in the testing of rodent responses to fear stimuli. Laboratory rodents are usually tested in spatially constrained arenas, where they are presented with a single proximal stimulus. Field or semi-captive studies normally occur at much larger spatial scales, and animals are exposed to varying environmental conditions as well as the specific stimulus of interest. These differences can have important implications to the interpretation of the responses measured (Lima, 1998; Lima and Bednekoff, 1999). Allenbyi's gerbils (*G. allenbyi*) and the greater Egyptian gerbil (*G. pyramidurn*) exposed to Barn owls (*Tyto alba)* at close proximity show a greater reduction in activity than when presented with the same predator in a semi-captive setting (Abramsky et al., 1996). Similarly, house mice (*M. musculus domesticus*) (Dickman, 1992), bank voles (*Clethrionomys glareolus*), and meadow voles (*Microtus pennsylvanicus*) (Perrot-Sinal et al., 1996, 1999, 2000) show a strong aversion and reduce activity when exposed to mustelid odor in relatively small enclosures. Conversely, in large scale enclosures, hairy-footed gerbils (*Gerbillurus tytonis*) (Hughes and Ward, 1993), gray-tail voles (*Microtus canicaudus*) and bank voles (*C. glareolus*) show little to no anti-predator response. This pattern is also observed in other small non-rodent mammals (Ward et al., 1997) and fish (Fraser and Huntingford, 1986; Irving and Magurran, 1997).

Kavaliers and Choleris (2001) suggested that the differences between laboratory and field results may be due to differences in the intensity of the cue. They argue that laboratory animals commonly experience single intense cues while animals in field experiments, although sometimes exposed to the same type of cues, can also assess predation risk by integrating several other relevant cues (e.g., habitat structure, vision, odors, sound) simultaneously (Kavaliers and Choleris, 2001). Thus, the cue of interest (i.e., the cue manipulated by the experimenter) is "drowned out" by the information contained in the other signals. Additionally, it has been suggested that very high predation risk may, counterintuitively, reduce some antipredator responses and increase others (e.g., vigilance vs. time allocation; Lima, 1998). For example, at very high levels of risk, animals are expected to completely avoid an area either by moving away (i.e., habitat partitioning) or by hiding until the risk is reduced (i.e., time partitioning). While at low levels of predation risk, animals may choose to remain in the area but increase vigilance instead (Lima, 1998). This has been demonstrated in desert rodents (Abramsky et al., 1996) and consistently in rats (Blanchard D.C et al., 1991; Blanchard et al., 1993, 1998).

Hence, if a pest rodent management strategy is to be effective, consideration of the intensity and the distribution of the predator cues is essential. Here, we suggest that utilizing a combination of

cues may prove more effective (e.g., predator odor paired with either predator call or conspecific alarm playback).

Fear Learning and Habituation

Before any animal can respond appropriately to reduce its risk of predation, it needs to be able to perceive and estimate such risk. Animals can use ultimate or proximate cues to estimate the risk of predation (Kavaliers and Choleris, 2001). Ultimate cues constitute the actual detection of a predator itself; these could be visual (Magurran and Girling, 1986; Atkins et al., 2017), auditory (Smith et al., 2017; Suraci et al., 2017b), or tactile (Kavaliers and Choleris, 2001). While proximate cues are commonly of a chemical nature, such as odors (Parsons et al., 2017). Ultimate cues convey immediate risk (i.e., the predator is present here right now), while proximate cues convey temporally dependent risk (Parsons and Blumstein, 2010; Parsons et al., 2017) (i.e., the predator was here sometime in the past; Parsons and Blumstein, 2010; Parsons et al., 2017). However, in most cases, these cues are encountered simultaneously—i.e., if an animal sees a predator, it can likely detect the predator by audition and olfaction. By separating the effects of different types of predator cues, each can convey different kinds of information—i.e., presence of a potential threat vs. identity of the threat (Blumstein et al., 2000; Mathis and Vincent, 2000). Ultimate cues of predation are potentially more reliable and convey a good estimate of the actual risk of predation. Proximate cues are more variable in nature, and can provide an underestimation of risk if predators are present, or an overestimation when they are absent (Lima and Dill, 1990; Abrams, 1994; Lima, 1998).

Once a prey animal detects a predator cue it must be able to recognize and assess the risk associated with it, leading to a fear response. In the brain, fear can be categorized in two distinct modes, innate and learnt (Canteras et al., 2015). Innate fear refers to the defensive response to aversive stimuli with no previous experience of such stimulus (Canteras et al., 2015; Parsons et al., 2017). Learned fear is the development of conditioned fear behaviors—i.e., defensive responses to a innocuous stimulus or context, shown after repeated pairings of the innocuous stimulus and an adverse one (Rescorla and Wagner, 1972).

Innate fear is also known as "species memory" or "phyletic memory" (Canteras et al., 2015), and it has been described in a myriad of different taxa: including invertebrates (Dalesman et al., 2007); fish (Berejikian et al., 2003; Vilhunen and Hirvonen, 2003); amphibians (Semlitsch and Reyer, 1992); birds (Veen et al., 2000; Göth, 2001); marsupials (Anson and Dickman, 2013), rabbits (Monclús et al., 2005), rodents (Dielenberg and McGregor, 2001; Bowen et al., 2012, 2013; Parsons et al., 2017); ungulates (Chamaillé-Jammes et al., 2014); and primates (Gould and Sauther, 2007). In mammals, the neurocircuitry that categorizes innate fear responses is initiated by an increase in Fos expression in the posteroventral part of the medial amygdalar (MEApv) and in the dorsomedial part of the ventromedial hypothalamic nucleus (VMHdm) (Pérez-Gómez et al., 2015). The activation of the dorsomedial and central divisions of the VMH (VMHdm/c) are linked with the initiation of a series of context-dependent somatomotor and autonomic defensive behaviors, including generalized passive hiding and freezing responses, as well as running and jumping (Wang et al., 2015). Innate fear response, and the initiation of autonomic defensive behaviors, could therefore be considered the "default" mechanistic mode of defense when exposed to a novel predator cue. However, the future fear response to the same cue is not always fixed and can be modulated by experience; this is the paradigm of "habituation" (Rankin et al., 2009; Blumstein, 2016). Habituation is the reduction of a natural response to a stimulus as a consequence of repetitive exposure (Davis, 1970; Staddon, 1993). This can represent a major obstacle in the application of anti-predator responses for wildlife management (Bomford and O'Brien, 1990; Koehler et al., 1990; Samia et al., 2015; Blumstein, 2016). Habituation occurs when short-term memory suppresses the natural response to a recent stimulus (Staddon, 1993). Yet, if an animal fails to respond to a stimulus that signals an increase in predation risk, that animal is bound to suffer predation. Thus, it raises questions as to why habituation is widespread among several different taxa and stimuli types (Davis, 1970; Williams et al., 1990; Talling et al., 1998; Nowak et al., 2014) and why animals are not fearful at all times. This is explained by the fact that antipredator responses can be expensive (Ylönen and Brown, 2007; Martin, 2011; LaManna and Martin, 2016), and that not all stimuli can be regarded as honest. There are fundamental differences between the "actual" risk of predation and the "perceived" risk of predation (Creel, 2018), and most species have the cognitive tools to reduce the chances of "false positives" by learning.

After estimating the level of risk, an animal must be able to respond to reduce that risk. There are several stages to the predation process, through which antipredator defenses can act to reduce risks. Prey can reduce the probability of: being detected by the predator; that detection will lead to an attack; that an attack will lead to death or serious injury; and being the individual that is killed (Hamilton, 1971; Turner and Pitcher, 1986; Uetz and Hieber, 1994). To achieve this, animals can: (i) avoid a high risk area (Schmitz et al., 1997; Ojeda and Muñoz, 1999; Wirsing et al., 2008; Mao et al., 2010); (ii) wait until a risk decreases to become active (Lima and Bednekoff, 1999; Kotler et al., 2002; Valeix et al., 2009); (iii) reduce foraging (Brown et al., 1988; Herman and Valone, 2000; Altendorf et al., 2001); (iv) increase vigilance (Childress and Lung, 2003; Cresswell et al., 2003; Fortin et al., 2004; Embar et al., 2011; Iribarren and Kotler, 2012); (v) discourage predation by direct signaling (FitzGibbon and Fanshawe, 1988), (vi) employ active defenses (Corcoran and Conner, 2012); and (vii) aggregate with con-specifics (Hamilton, 1971; Pulliam, 1973; Bowen et al., 2013). In natural systems, these strategies are usually effective in unison (Kotler et al., 2010). Thus, predator defenses can be behavioral (Lima, 1990; Altendorf et al., 2001; Abramsky et al., 2002), morphological (Agrawal and Fishbein, 2006), physiological (Lima, 1998; van Donk et al., 1999), or ecological (Ojeda and Muñoz, 1999; Wirsing et al., 2008; Mao et al., 2010). These defenses are associated with non-consumptive costs and, in order to remain in the population, these costs need to be offset by a reduction in the "actual" risk of predation (Creel, 2018); these costs to risks ratio is what drives habituation.

Laboratory rodents demonstrate defensive responses to predators without previous experience (Parsons et al., 2017).

However, this response is not unchallengeable, as prolonged exposure to predator cues reduces anti-predator behavior in laboratory rats (Williams et al., 1990). Moreover, it is well-understood that varying levels of predation risk can shape wild rodent anti-predator behavior (Brown et al., 1999; Ylönen and Brown, 2007). Thus, rodent antipredator responses seem to be non-binary (i.e., not simply "on and off"), and are shaped by fear learning (Staples et al., 2005).

Fear learning or contextual conditioning involves a different, although related pathway to innate fear neurocircuitry. The current working model for fear learning involves sequential activation, signaling, and feedback, primarily between four brain regions well-characterized for their role in fear acquisition and consolidation (McNally et al., 2011). This model describes how fear learning occurs when the difference between the actual vs. the expected intensity of a aversive unconditioned stimulus is encoded by an error signal (Rescorla and Wagner, 1972). McNally et al. (2011) it mostly involves conditioning with shock rather than with predator-related cues. However other studies have looked at conditioning with cat odor (e.g., Staples et al., 2005). When rats were placed in a context where they previously encountered a predator odor they displayed brain activation in a subset of the regions activated by the predator odor itself: this included the dorsal premammillary nucleus, ventrolateral periaqueductal gray, cuneiform nucleus and locus ceruleus (Staples et al., 2005). Little activation was seen in the amygdala or hippocampus. These results show that stimuli associated with predatory threat come to activate similar brain regions to the threat stimulus itself. If an animal experiences an unexpected aversive stimulus (e.g., predator attack), then the actual intensity of the stimulus will be higher than the expected, thus the stimulus would drive fear learning. Conversely, if an aversive stimulus—e.g., predator attack—was expected—e.g., encounter with a predator cue –, then the expected and actual intensity of aversive stimulus will match, and fear learning does not occur. Finally, when the actual intensity of the aversive stimulus is lower than the expected intensity—e.g., perceived predator cue but predator does not attack– then fear learning extinction occurs—i.e., habituation (Schaller, 1972; McNally et al., 2011).

In mammals, fear learning involves complex neural circuitry within the amygdala (McNally et al., 2011). When an aversive stimulus is detected (e.g., predator attack), this activates spinal and trigeminal dorsal horn neurons that project to the midbrain periaqueductal gray (PAG; McNally et al., 2011). Signaling then travels from the PAG, through the midline and intralaminar thalamus, to the dorsomedial prefrontal cortex (dmPFC) and lateral amygdala (LA) depolarizing pyramidal neurons (McNally et al., 2011). Associative fear learning is achieved when the co-occurrence of the aversive stimulus (e.g., predator attack) with an associated stimulus (e.g., predator cue) strengthen the thalamic and cortical afferent inputs to LA through N-methyl-D-aspartate receptor (NMDA)-mediated long term potentiation (McNally et al., 2011). Consequently, future exposure to the associated stimulus (e.g., predator cue) activates LA projection neurons to the central amygdala (CeA), leading to inhibition of the ventrolateral PAG (vlPAG), and inciting an antipredator response (McNally et al., 2011). Repeated exposures to the associated stimulus alone causes weakening of the auditory thalamic and cortical afferent inputs to LA pyramidal neurons through long term depression at NMDA receptors (McNally et al., 2011).

Learning aids in the recognition of threats, but through conditioning, it is the mechanism by which animals also estimate predation risk (Bolles, 1970; Crawford and Masterson, 1982; Cook and Mineka, 1990; Curio, 1993). There are many examples of different taxa that show certain innate responses to predators: from finches (Curio, 1993), moose (Berger et al., 2001), black-tail deer (Chamaillé-Jammes et al., 2014), Hokkaido deer (Osada et al., 2014), rabbits (Monclús et al., 2005), deer mice (Coss, 1999), ground squirrels (Hirsch and Bolles, 1980), and laboratory mice (Pérez-Gómez et al., 2015). But, more importantly, these innate predator responses can be enhanced or modified through fear learning (Berger et al., 2001). However, individual fear learning requires an animal to experience an adverse predator encounter directly, which may not be conducent to future survival. Thus, to acquired "knowledge" putatively through conspecifics is more advantageous (Russon, 1997).

Social learning occurs when an animal acquires information (i.e., the observer) by witnessing the actions of another, more experienced individual (i.e., the demonstrator; Heyes, 1994; Choleris and Kavaliers, 1999). The first taxa where social learning was characterized was fish (Von Frisch, 1942). Nowadays, social learning has been reported not only in other fish species (Chivers et al., 1995; Mirza and Chivers, 2000; Brown and Laland, 2005), but also birds (Curio et al., 1978; Curio, 1988; Magurran, 1989; Martínez et al., 2017); marsupials (Griffin and Evans, 2003); ungulates (Berger et al., 2001); primates (Cook et al., 1985; Bartecki and Heymann, 1987; Mineka and Cook, 1988; Cook and Mineka, 1990; Srivastava, 1991); laboratory rats (Lore et al., 1971); and mice (Kavaliers et al., 2001a,b; Sanders et al., 2013).

Both individual and social fear learning are widely used in wildlife management programs with species conservation goals (Griffin et al., 2001), yet has been somewhat neglected in the application of fear as a management tool. Schulte (2016) argues that when using artificial predator cue to alter the perceived predation risk by pest animals, a Batesian mimicry type dilemma is difficult to avoid. Batesian mimicry occurs when a non-dangerous species mimics the appearance, smell and/or behavior of a co-occurring dangerous species, thereby protecting itself from the attack of predators that have learned to avoid the dangerous species (Bates, 1861). This type of mimicry is maintained only when the relative frequency of the dangerous species is higher than that of the mimic, thus the predator maintains a conditioned avoidance to the mimic (Duncan and Sheppard, 1965). This argument also brings forward the effect of tolerance as another factor affecting fear response. In a comprehensive review, Blumstein (2016) stablished a clear difference between habituation and tolerance. Habituation is a process that acts at the scale of the individual, with each animal modifying their response to different stimulus based on their associated consequences across repeated exposures (Rankin et al., 2009; Blumstein, 2016). Tolerance, also involving a reduced response to a stimulus, and can emerge through habituation-like processes but also through other ecological pressures (e.g., competition; Owens, 1977; Blumstein, 2016).

Moreover, tolerance through habituation can be transferable to different stimulus—e.g., squirrels inhabiting urban areas reduced their anti-predator response to foxes (Mccleery, 2009).

In a pest management context, the widespread deployment of predator cues (i.e., mimics) in the environment has the potential to overcome the relative frequency of the real predator, thus fear conditioning is lost (Schulte, 2016). Moreover, we would expect pest rodent populations inhabiting urban areas, would be more tolerant to human disturbances, and this tolerance may translate to a reduction in their antipredator response (Mccleery, 2009; Blumstein, 2016). A way of overcoming this dilemma is to maintain conditioning by aversive reinforcement (Kloppers et al., 2005; Leigh and Chamberlain, 2008; Cromsigt et al., 2013). These aversive reinforcements can be administered repeatedly (Huang et al., 1992; Dunsmoor et al., 2007) or be of high intensity (Abrams, 1994; Siegmund and Wotjak, 2007). However, this can be logistically difficult and undesirable (Baruch-Mordo et al., 2011). The implementation of repeated exposures of an intense aversive stimulus can be expensive, has negative animal welfare implications, or be lethal to the target and non-target species (Schulte, 2016). The question then becomes, what aversive reinforcement is required to maintain fear conditioning? One possible solution is to use predator cues that not only incite fear, but can also cause long-term anxiety (Schakner and Blumstein, 2013). This type of response to repeated stimulus is called sensitization and has been reported in seals (Götz and Janik, 2011, 2015), ungulates (Cox et al., 2012), and marsupials (Parsons and Blumstein, 2010). However, to date there is no evidence that rodents would show sensitization to predator cues.

Information Gathering

In a comprehensive review of the role of predator odor in predator-prey interactions, Parsons et al. (2017) suggests that the attraction of prey to some predator smells may be due prey species gathering information either on the identity or temporal characteristics of the scent (Parsons et al., 2017). When an animal approaches the scent of a predator—i.e., predator inspection—it does so in order to obtain information about the actual risk of predation (Fishman, 1999). The animal may gain certain benefits from doing so, namely (i) acquiring information about the nature of the potential threat; (ii) informing conspecifics of the potential threat; (iii) deterring predator attack; and (iv) possibly even advertising one's quality to mates (Dugatkin and Godin, 1992).

Parsons et al. (2017) argues that this phenomenon can confound interpretation of empirical studies testing the repellent potential of predator scents and, as exemplar, describe how hairy-nosed wombats (*Lasiorhinus latifrons*), when presented with dingo (*Canis dingo*) scents, remained within 200 m of the stimulus (Sparrow et al., 2016). However, while still present in the area, wombats stopped their normal digging behavior, thus there was a significant reduction of their impact to human activity (Sparrow et al., 2016).

With the exception of pheromones (i.e., single species communication molecules), most scents are "dose-dependent" (Glimcher, 2010; Vasudevan and Vyas, 2013). Higher concentrations of a specific predator scent may convey higher or lower predation risk (Schmeisser et al., 2013). However, composition is also important. Scherer and Smee (2016), suggested that most prey species are sensitive to their predators' diet. For example, dwarf hamsters (*Phodopus campelli*) show a stronger anti-predator response when predators fed upon conspecifics (Apfelbach et al., 2015).

Another aspect of information gathering in predator cue inspection is the temporal component. Predator scent is normally complex, and composed of a myriad of different molecules, with different characteristics (Parsons et al., 2017). Each component within this complex mixture react to environmental conditions (e.g., bacterial decomposition, UV light) at different rates and in different manners (Rasmussen, 1988; Muller-Schwarze, 2006), ultimately modifying the chemical profile of the cue over time. Even handling and storage of the chemical cues can alter the way animals perceive them (Hoffmann et al., 2009; Hegab et al., 2014). It is therefore possible that changes in chemical structure of the signal may also change its information over time (Parsons and Blumstein, 2010). Older cues may convey that the predator is not there, and left a long time ago, while fresher cues can convey immediate danger (Hurst and Beynon, 2004; Parsons et al., 2017). However, there is some evidence that these changes may not affect rat responses (*Rattus* spp.) (Bytheway et al., 2013). Alternatively, animals may approach older odor cues since obtaining information from degraded scent may be more difficult, since only the non-volatile components remain (Parsons et al., 2017).

This raises the question; how can predator cues be applied in a rodent management context? The use of predator scent as a rodent management tool requires a better understanding on how these types of cues work. Just as adverse reinforcement is used to prevent habituation, the repeated application of odor cues to maintain their freshness can be logistically expensive and difficult. Current evidence suggest that wild rodents are not affected by aging scent (Bytheway et al., 2013), however, it is important to note that this is based on 1-day old scent. A better understanding is required when, along the aging time-line, anti-predator response disappears. This can help determine the rate at which the scent needs to be re-applied. Note, this is an issue specific to the use of chemical cues. Visual and auditory stimuli (or their pairing with chemical cues), might prove to be more effective.

CONCLUSIONS AND IMPLICATIONS FOR RODENT MANAGEMENT

Pest rodent species adaptability is what has placed them in constant conflict with humans. For humans, rodent populations' cost money, damage buildings, eat crops and transmit diseases. In contrast, rodents are "fighting" for their lives. Thus, it is not surprising that this constant struggle for survival pushes mice and rats to risk safety for food, and has made them very capable of assessing risks across very fine scales.

To use anti-predator responses as a rodent management tool, we need to follow a holistic approach. First, we need to understand that these animal's motivations are strong, thus our

strategy needs to be stronger. It is essential to reinforce our approaches. Using a combination of predator and conspecific cues (e.g., predator odor, paired with predator models, and playbacks of both the predator and conspecifics alarm calls) to simulate high predation risk, but also maintaining the actual risks as high (e.g., repeated simultaneous aversive stimuli), could prevent most of the issues discussed in this review. For example, in agricultural systems, a sentinel system can be applied, where a crop area can be heavily guarded with simulated predators, while another is not, however treated with rodenticide. This can increase rodenticide intake, while reducing widespread use.

However, these are not easy tasks, and economic, logistical, and ethical costs need to be addressed. Yet, some steps in the right direction have been made in other systems. For example, Cromsigt et al. (2013) proposed the use of more intense methods of hunting ungulates (e.g., using dogs, targeting females with calves) in order to induce fear and deter these animals from areas of interest. Regardless of the polemic animal welfare implications, this proposal aims at increasing the intensity of the perceived risk (Cromsigt et al., 2013). Suraci et al. (2017a) have developed a motion triggered systems to playback predator sounds only when an animal approaches the devices, the cue is only used at proximity, intensifying the fear response. The effectiveness of this device has been demonstrated with pumas (*Puma concolor*) (Smith et al., 2017), and raccoons (*Procyon lotor*) (Suraci et al., 2016). One point of caution is the long term consequences of simulated high predation risk deployment are not fully understood, and some have raised doubts about how high this perceived risk should be (Duffield et al., 2017; Haapakoski et al., 2018). Sustained perception of very high risks of predation can drive a cycle of dynamic terminal investment (Duffield et al., 2017), that can have the desired short term deterrent effects, but produce long term population increases.

There are also synergies that can be achieved, if we consider the biological nature of the fear response. For example, the modification of antipredator behavior by *T. gondii* infections could assist with the reduction on the prevalence of *T. gondii*, at the community level, as a tool in conservation programs (Dubey and Crutchley, 2008; Fancourt and Jackson, 2014; Fancourt et al., 2014).

The theoretical framework of using fear as a way of managing pest rodent populations is sound, but it is not in any way simple. Pest rodents, either native or introduced, are embedded within a dynamic ecological system. If anti-predator responses are to be used as a pest management tool, it is essential to recognize that rodent's anti-predator responses are non-binary and rely on complex contextual cues.

AUTHOR CONTRIBUTIONS

MB-P conceived and wrote the initial manuscript. KS assisted with writing of particular sections of the manuscript. RK, JL, MC, and IM offered editorial advice and help structure the manuscript. All authors discussed the ideas presented and contributed to the final manuscript.

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REFERENCES

Abel, E. L. (1991). Alarm substance emitted by rats in the forced-swim test is a low volatile pheromone. *Physiol. Behav.* 50, 723–727.

Abrams, P. A. (1994). Should prey overestimate the risk of predation? *Am. Nat.* 144, 317–328.

Abramsky, Z., Rosenzweig, M. L., and Subach, A. (2002). The cost of apprehensive foraging. *Ecology* 83, 1330–1340. doi: 10.2307/3071947

Abramsky, Z., Strauss, E. G., Subach, A., Riechman, A., and Kotler, B. P. (1996). The effect of barn owls (*Tyto alba*) on the activity and microhabitat selection of *Gerbillus allenby*; and *G. pyramidum*. *Oecologia* 105, 313–319.

Abu Baker, M. A., and Brown, J. S. (2012). Patch use behaviour of *Elephantulus myurus* and *Micaelamys namaquensis*: the role of diet, foraging substrates and escape substrates. *Afr. J. Ecol.* 50, 167–175. doi: 10.1111/j.1365-2028.2011.01309.x

Agrawal, A. A., and Fishbein, M. (2006). Plant defense syndromes. *Ecology* 87, S132–S149. doi: 10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2

Albert, C. A., Wilson, L. K., Mineau, P., Trudeau, S., and Elliott, J. E. (2010). Anticoagulant rodenticides in three owl species from western Canada, 1988–2003. *Arch. Environ. Contam. Toxicol.* 58, 451–459. doi: 10.1007/s00244-009-9402-z

Allmann-lselin, I. (2000). "*4*. Husbandry," in *The Laboratory rat Handbook of Experimental Animals,* eds G. J. Krinke, G. R. Bullock, and T. Bunton (London; San Diego, CA: Academic Press, Inc.), 45–55. doi: 10.1016/B978-0-12-426400-7.X5037-7

Almeida, A., Corrigan, R. M., and Sarno, R. (2013). The economic impact of commensal rodents on small businesses in Manhattan's Chinatown: trends and possible causes. *Suburb. Sustain.* 1, 1–15. doi: 10.5038/2164-0866.1.1.2

Altendorf, K. B., Laundr,é, J. W., López González, C. A., and Brown, J. S. (2001). Assessing effects of predation risk on foraging behavior of mule deer. *J. Mammal.* 82, 430–439. doi: 10.1644/1545-1542(2001)082<0430:aeopro>2.0.co;2

Anson, J. R., and Dickman, C. R. (2013). Behavioral responses of native prey to disparate predators: naiveté and predator recognition. *Oecologia* 171, 367–77. doi: 10.1007/s00442-012-2424-7

Apfelbach, R., Blanchard, D. C., Blanchard, R. J., Hayes, R. A., and McGregor, I. S. (2005). The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neurosci. Biobehav. Rev.* 29, 1123–1144. doi: 10.1016/j.neubiorev.2005.05.005

Apfelbach, R., Parsons, M. H., Soini, H. A., and Novotny, M. V. (2015). Are single odorous components of a predator sufficient to elicit defensive behaviors in prey species? *Front. Neurosci.* 9:263. doi: 10.3389/fnins.2015.00263

Atkins, A., Redpath, S. M., Little, R. M., and Amar, A. (2017). Experimentally manipulating the landscape of fear to manage problem animals. *J. Wildl. Manage.* 81, 610–616. doi: 10.1002/jwmg.21227

Atkinson, I. A. E. (1985). "The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifaunas," in *Conservation of Island Birds: Case Studies for the Management of Threatened Island Species*, ed P. Moors (Cambridge: ICBP Technical Publication), 35–81.

Atkinson, U. A. E. (1973). Spread of the ship rat (*Rattus r. rattus* L.) III New Zealand. *J. R. Soc. New Zeal.* 3, 457–472.

Ayral, F., Artois, J., Zilber, A.-L., Widen, F., Pounder, K. C., Aubert, D., et al. (2015). The relationship between socioeconomic indices and potentially zoonotic pathogens carried by wild Norway rats: a survey in Rhône, France (2010–2012). *Epidemiol. Infect.* 143, 586–599. doi: 10.1017/S0950268814001137

Banks, P. B. (1998). Responses of Australian bush rats, *Rattus fuscipes*, to the odor of introduced *Vulpes vulpes*. *J. Mammal.* 79, 1260–1264.

Barnett, S. A. (1958). Experiments on "neophobia" in wild and laboratory rats. *Br. J. Psychol.* 49, 195–201.

Barnett, S. A. (2001). *The Story of Rats: Their Impact on us, and Our Impact on Them*. Crows Nest, NSW: Allen & Unwin.

Barnett, S. A., and Cowan, P. E. (1976). Activity, exploration, curiosity and fear: an ethological study. *Interdiscip. Sci. Rev.* 1, 43–62.

Bartecki, U., and Heymann, E. W. (1987). Field observation of snake-mobbing in a group of saddle-back tamarins, *Saguinus fuscicollis nigrifrons*. *Folia Primatol.* 48, 199–202.

Baruch-Mordo, S., Breck, S. W., Wilson, K. R., and Broderick, J. (2011). The Carrot or the stick? Evaluation of education and enforcement as management tools for human-wildlife conflicts. *PLoS ONE* 6:e15681. doi: 10.1371/journal.pone.0015681

Bates, H. W. (1861). XXXII. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidæ. *Trans. Linn. Soc. London* 23, 495–566.

Bedoya-Pérez, M. A., Carthey, A. J. R., Mella, V. S. A., McArthur, C., and Banks, P. B. (2013). A practical guide to avoid giving up on giving-up densities. *Behav. Ecol. Sociobiol.* 67, 1–13. doi: 10.1007/s00265-013-1609-3

Bennett, G. W., Owens, J. M., and Corrigan, R. M. (2003). *Truman's Scientific Guide to Pest Management Operations*. 6th Edn. Duluth, MN: Purdue University.

Berardi, N., and Maffei, L. (1999). From visual experience to visual function: Roles of neurotrophins. *J. Neurobiol.* 41, 119–126.

Berdoy, M., and Macdonald, D. W. (1991). Factors affecting feeding in wild rats. *Acta Oecol.* 12, 261–279.

Berdoy, M., Webster, J. P., and Macdonald, D. W. (1995a). Parasite-altered behaviour: is the effect of *Toxoplasma gondii* on *Rattus norvegicus* specific? *Parasitology* 111(Pt 4), 403–409. doi: 10.1017/S0031182000065902

Berdoy, M., Webster, J. P., and Macdonald, D. W. (1995b). The manipulation of rat behaviour by *Toxoplasma gondii*. *Mammalia* 59, 605–614. doi: 10.1515/mamm.1995.59.4.605

Berdoy, M., Webster, J. P., and Macdonald, D. W. (2000). Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc. R. Soc. London. Ser. B Biol. Sci.* 267, 1591–1594. doi: 10.1098/rspb.2000.1182

Berejikian, B. A., Tezak, E. P., and LaRae, A. L. (2003). Innate and enhanced predator recognition in hatchery-reared chinook salmon. *Environ. Biol. Fishes* 67, 241–251. doi: 10.1023/A:1025887015436

Berger, J., Swenson, J. E., and Persson, I.-L. (2001). Recolonizing carnivores and naïve prey: conservation lessons from Pleistocene extinctions. *Science* 291, 1036–1039. doi: 10.1126/science.1056466

Berger-Tal, O., and Kotler, B. P. (2010). State of emergency: Behavior of gerbils is affected by the hunger state of their predators. *Ecology* 91, 593–600. doi: 10.1890/09-0112.1

Berger-Tal, O., Mukherjee, S., Kotler, B. P., and Brown, J. S. (2010). Complex state-dependent games between owls and gerbils. *Ecol. Lett.* 13, 302–310. doi: 10.1111/j.1461-0248.2010.01447.x

Beverley, J. K. (1976). Toxoplasmosis in animals. *Vet. Rec.* 99, 123–127.

Beverley, J. K. A. (1959). Congenital transmission of *Toxoplasmosis* through successive generations of mice. *Nature* 183, 1348–1349. doi: 10.1038/1831348a0

Blanchard, D. C., Blanchard, R. J., and Rodgers, R. J. (1991). "Risk assessment and animal models of anxiety," in *Animal Models in Psychopharmacology*, eds B. Olivier, J. Mos, and J. L. Slangen (Basel: Birkhäuser Basel), 117–134.

Blanchard, R. J., Blanchard, D. C., Agullana, R., and Weiss, S. M. (1991). Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiol. Behav.* 50, 967–972.

Blanchard, R. J., Nikulina, J. N., Sakai, R. R., McKittrick, C., McEwen, B., and Blanchard, D. C. (1998). Behavioral and endocrine change following chronic predatory stress. *Physiol. Behav.* 63, 561–569.

Blanchard, R. J., Yudko, E. B., Rodgers, R. J., and Blanchard, D. C. (1993). Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behav. Brain Res.* 58, 155–165.

Bleicher, S. S. (2017). The landscape of fear conceptual framework: definition and review of current applications and misuses. *PeerJ.* 5:e3772. doi: 10.7717/peerj.3772

Blumstein, D. T. (2016). Habituation and sensitization: new thoughts about old ideas. *Anim. Behav.* 120, 255–262. doi: 10.1016/j.anbehav.2016.05.012

Blumstein, D. T., Daniel, J. C., Griffin, A. S., and Evans, C. S. (2000). Insular tammar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. *Behav. Ecol.* 11, 528–535. doi: 10.1093/beheco/11.5.528

Bohls, P., and Koehnle, T. J. (2017). Responses of eastern gray squirrels (*Sciurus carolinensis*) to predator calls and their modulation by coat color. *Am. Midl. Nat.* 178, 226–236. doi: 10.1674/0003-0031-178.2.226

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychol. Rev.* 77, 32–48.

Bomford, M., and O'Brien, P. H. (1990). Sonic deterrents in animal damage control: a review of device tests and effectiveness. *Wildl. Soc. Bull.* 18, 411–422.

Bonnington, C., Gaston, K. J., and Evans, K. L. (2013). Fearing the feline: domestic cats reduce avian fecundity through trait-mediated indirect effects that increase nest predation by other species. *J. Appl. Ecol.* 50, 15–24. doi: 10.1111/1365-2664.12025

Bowen, M. T., Keats, K., Kendig, M. D., Cakic, V., Callaghan, P. D., and McGregor, I. S. (2012). Aggregation in quads but not pairs of rats exposed to cat odor or bright light. *Behav. Processes* 90, 331–336. doi: 10.1016/j.beproc.2012.03.014

Bowen, M. T., Kevin, R. C., May, M. D., Staples, L. G., Hunt, G. E., and McGregor, I. S. (2013). Defensive aggregation (huddling) in *Rattus norvegicus* toward predator odor: individual differences, social buffering effects and neural correlates. *PLoS ONE* 8:e68483. doi: 10.1371/journal.pone.0068483

Bramley, G. N., and Waas, J. R. (2001). Laboratory and field evaluation of predator odors as repellents for kiore (*Rattus exulans*) and ship rats (*R. rattus*). *J. Chem. Ecol.* 27, 1029–1047. doi: 10.1023/a:1010399322861

Brechbühl, J., Klaey, M., and Broillet, M.-C. (2008). Grueneberg ganglion cells mediate alarm pheromone detection in mice. *Science* 321, 1092–1095. doi: 10.1126/science.1160770

Brechbühl, J., Moine, F., Klaey, M., Nenniger-Tosato, M., Hurni, N., Sporkert, F., et al. (2013). Mouse alarm pheromone shares structural similarity with predator scents. *Proc. Natl. Acad. Sci. U.S.A.* 110, 4762–4767. doi: 10.1073/pnas.1214249110.

Brown, C., and Laland, K. (2005). Social learning and life skills training for hatchery reared fish. *J. Fish Biol.* 59, 471–493. doi: 10.1111/j.1095-8649.2001.tb02354.x

Brown, J. S., and Alkon, P. U. (1990). Testing values of crested porcupine habitats by experimental food patches. *Oecologia* 83, 512–518.

Brown, J. S., Kotler, B. P., Smith, R. J., and Wirtz, I. I., W. O. (1988). The effects of owl predation on the foraging behavior of heteromyid rodents. *Oecologia* 76, 408–415.

Brown, J. S., Laundré, J. W., and Gurung, M. (1999). The ecology of fear: Optimal foraging, game theory, and trophic interactions. *J. Mammal.* 80, 385–399.

Brown, J. S., and Morgan, R. A. (1995). Effects of foraging behavior and spatial scale on diet selectivity: a test with fox squirrels. *Oikos* 74, 122–136.

Brudzynski, S. M., and Chiu, E. M. C. (1995). Behavioural responses of laboratory rats to playback of 22 kHz ultrasonic calls. *Physiol. Behav.* 57, 1039–1044.

Brunton, C. F. A., Macdonald, D. W., and Buckle, A. P. (1993). Behavioural resistance towards poison baits in brown rats, *Rattus norvegicus*. *Appl. Anim. Behav. Sci.* 38, 159–174.

Buckle, A. P. (2013). Anticoagulant resistance in the United Kingdom and a new guideline for the management of resistant infestations of Norway rats (*Rattus norvegicus* Berk.). *Pest Manag. Sci.* 69, 334–341. doi: 10.1002/ps.3309

Buckle, A. P., and Smith, R. H. (2015). *Rodent Pests and Their Control*. 2nd Edn. Oxfordshire; Boston, MA: CABI.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D., and Sullivan, T. P. (1998). Field testing synthetic predator odors for roof rats (*Rattus rattus*) in Hawaiian macadamia nut orchards. *J. Chem. Ecol.* 24, 603–630.

Bytheway, J. P., Carthey, A. J. R., and Banks, P. B. (2013). Risk vs. reward: how predators and prey respond to aging olfactory cues. *Behav. Ecol. Sociobiol.* 67, 715–725. doi: 10.1007/s00265-013-1494-9

Campeau, S., and Davis, M. (1995). Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured

with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J. Neurosci.* 15(3 Pt 2), 2312–2327.

Canteras, N., Pavesi, E., and Carobrez, A. (2015). Olfactory instruction for fear: neural system analysis. *Front. Neurosci.* 9:276. doi: 10.3389/fnins.2015.00276

Capizzi, D., Bertolino, S., and Mortelliti, A. (2014). Rating the rat: global patterns and research priorities in impacts and management of rodent pests. *Mamm. Rev.* 44, 148–168. doi: 10.1111/mam.12019

Caro, T. (2005). *Antipredator Defenses in Birds and Mammals*. Chicago, IL: University of Chicago Press.

Carthey, A. J. R., and Banks, P. B. (2016). Naiveté is not forever: responses of a vulnerable native rodent to its long term alien predators. *Oikos* 125, 918–926. doi: 10.1111/oik.02723.

Chamaillé-Jammes, S., Malcuit, H., Le Saout, S., and Martin, J.-L. (2014). Innate threat-sensitive foraging: black-tailed deer remain more fearful of wolf than of the less dangerous black bear even after 100 years of wolf absence. *Oecologia* 174, 1151–1158. doi: 10.1007/s00442-013-2843-0

Chamero, P., Leinders-Zufall, T., and Zufall, F. (2012). From genes to social communication: molecular sensing by the vomeronasal organ. *Trends Neurosci.* 35, 597–606. doi: 10.1016/j.tins.2012.04.011

Charnov, E. L. (1979). *Optimal Foraging: Some Thoretical Explorations*. Salt Lake City, UT: Center of Quantitative Science, University of Washington, Seatle. Institute of Animal Resource Ecology, University of British Columbia Vancouver.

Childress, M. J., and Lung, M. A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Anim. Behav.* 66, 389–398. doi: 10.1006/anbe.2003.2217

Chitty, D. (1954). *Control of Rats and Mice*, eds D. Chitty and H. N. Southern. Oxford; Chicago, IL: Oxford: Clarendon Press.

Chivers, D. P., Brown, G. E., and Smith, R. J. F. (1995). Acquired recognition of chemical stimuli from pike, *Esox lucius*, by Brook sticklebacks, *Culaea inconstans* (Osteichthyes, Gasterosteidae). *Ethology* 99, 234–242.

Choleris, E., and Kavaliers, M. (1999). Social learning in animals: sex differences and neurobiological analysis. *Pharmacol. Biochem. Behav.* 64, 767–776.

Clinchy, M., Sheriff, M. J., and Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Funct. Ecol.* 27, 56–65. doi: 10.1111/1365-2435.12007

Clutton-Brock, J. (1999). *A Natural History of Domesticated Mammals*. 2nd Edn. Cambridge: Cambridge University Press.

Coeurdassier, M., Fritsch, C., Jacquot, M., van den Brink, N. W., and Giraudoux, P. (2018). "Spatial dimensions of the risks of rodenticide use to non-target small mammals and applications in spatially explicit risk modeling," in *Anticoagulant Rodenticides and Wildlife*, eds N. W. van den Brink, J. E. Elliott, R. F. Shore, and B. A. Rattner (Cham: Springer International Publishing), 195–227.

Cook, M., and Mineka, S. (1990). Selective associations in the observational conditioning of fear in rhesus monkeys. *J. Exp. Psychol. Anim. Behav. Process.* 16, 372. doi: 10.1037/0097-7403.16.4.372

Cook, M., Mineka, S., Wolkenstein, B., and Laitsch, K. (1985). Observational conditioning of snake fear in unrelated rhesus monkeys. *J. Abnorm. Psychol.* 94, 591–610. doi: 10.1037/0021-843X.94.4.591

Corcoran, A. J., and Conner, W. E. (2012). Sonar jamming in the field: effectiveness and behavior of a unique prey defense. *J. Exp. Biol.* 215, 4278–4287. doi: 10.1242/jeb.076943

Coss, R. G. (1999). "Effects of relaxed natural selection on the evolution," in *Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms*, eds S. A. Foster and J. A. Endler (New York, NY; Oxford: Oxford University Press on Demand), 180–208.

Coss, R. G., and Ramakrishnan, U. (2000). Perceptual aspects of leopard recognition by wild bonnet macaques (*Macaca radiata*). *Behaviour* 137, 315–335. doi: 10.1163/156853900502105

Cox, T. E., Murray, P. J., Hall, G. P., and Li, X. (2012). Manipulating resource use by goats with predator fecal odors. *Wildl. Soc. Bull.* 36, 802–806. doi: 10.1002/wsb.215

Crawford, M., and Masterson, F. A. (1982). Species-specific defense reactions and avoidance learning. *Pavlov. J. Biol. Sci. Off. J. Pavlov.* 17, 204–214.

Creel, S. (2018). The control of risk hypothesis: reactive vs. proactive antipredator responses and stress-mediated vs. food-mediated costs of response. *Ecol. Lett.* 21, 947–956. doi: 10.1111/ele.12975

Creel, S., Christianson, D., Liley, S., and Winnie, J. A. J. (2007). Predation risk affects reproductive physiology and demography of elk. *Science* 315:960. doi: 10.1126/science.1135918

Cresswell, W., Lind, J., Kaby, U., Quinn, J. L., and Jakobsson, S. (2003). Does an opportunistic predator preferentially attack nonvigilant prey? *Anim. Behav.* 66, 643–648. doi: 10.1006/anbe.2003.2233

Cromsigt, J. P. G. M., Kuijper, D. P. J., Adam, M., Beschta, R. L., Churski, M., Eycott, A., et al. (2013). Hunting for fear: innovating management of human-wildlife conflicts. *J. Appl. Ecol.* 50, 544–549. doi: 10.1111/1365-2664.12076

Curio, E. (1988). "Cultural transmission of enemy recognition," in *Social Learning: Psychological and Biological Perspectives Comparative Cognition and Neuroscience Series*, eds T. R. Zentall and B. G. Galef (Lawrence Erlbaum Associates, Inc.), 75–97. Available online at: https://books.google.com.au/books?id=mqFcAgAAQBAJ

Curio, E. (1993). "Proximate and developmental aspects of antipredator behavior," in *Advances in the Study of Behavior*, eds P. J. B. Slater, J. S. Rosenblatt, C. T. Snowdon, and M. Milinski (San Diego, CA: Academic Press, Inc.), 135–238.

Curio, E., Ernst, U., and Vieth, W. (1978). Cultural transmission of enemy recognition: one function of mobbing. *Science* 202, 899–901.

Dalesman, S., Rundle, S. D., and Cotton, P. A. (2007). Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. *Freshw. Biol.* 52, 2134–2140. doi: 10.1111/j.1365-2427.2007.01843.x

Dass, S. A. H., Vasudevan, A., Dutta, D., Soh, L. J. T., Sapolsky, R. M., and Vyas, A. (2011). Protozoan parasite *Toxoplasma gondii* manipulates mate choice in rats by enhancing attractiveness of males. *PLoS ONE* 6:e27229. doi: 10.1371/journal.pone.0027229

Davidson, D. L., and Morris, D. W. (2001). Density-dependent foraging effort of deer mice (*Peromyscus maniculatus*). *Funct. Ecol.* 15, 575–583. doi: 10.1046/j.0269-8463.2001.00569.x

Davis, M. (1970). Effects of interstimulus interval length and variability on startle-response habituation in the rat. *J. Comp. Physiol. Psychol.* 72, 177–192.

Dewan, A., Pacifico, R., Zhan, R., Rinberg, D., and Bozza, T. (2013). Non-redundant coding of aversive odours in the main olfactory pathway. *Nature* 497, 486–489. doi: 10.1038/nature12114

Dickman, C. R. (1992). Predation and habitat shift in the House mouse, *Mus domesticus*. *Ecology* 73, 313–322.

Dielenberg, R. A., and McGregor, I. S. (2001). Defensive behavior in rats towards predatory odors: a review. *Neurosci. Biobehav. Rev.* 25, 597–609. doi: 10.1016/S0149-7634(01)00044-6

Domjan, M. (1975). Poison-induced neophobia in rats: role of stimulus generalization of conditioned taste aversions. *Anim. Learn. Behav.* 3, 205–211.

Dowding, C. V., Shore, R. F., Worgan, A., Baker, P. J., and Harris, S. (2010). Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). *Environ. Pollut.* 158, 161–166. doi: 10.1016/j.envpol.2009.07.017

Dubey, J. P., and Crutchley, C. (2008). Toxoplasmosis in Wallabies (Macropus rufogriseus and Macropus eugenii): Blindness, Treatment with Atovaquone, and Isolation of *Toxoplasma gondii*. *J. Parasitol.* 94, 929–933. doi: 10.1645/GE-1448.1

Dubey, J. P., and Jones, J. L. (2008). *Toxoplasma gondii* infection in humans and animals in the United States. *Int. J. Parasitol.* 38, 1257–1278. doi: 10.1016/j.ijpara.2008.03.007

Dudeck, B. P., Clinchy, M., Allen, M. C., and Zanette, L. Y. (2018). Fear affects parental care, which predicts juvenile survival and exacerbates the total cost of fear on demography. *Ecology* 99, 127–135. doi: 10.1002/ecy.2050

Duffield, K. R., Bowers, E. K., Sakaluk, S. K., and Sadd, B. M. (2017). A dynamic threshold model for terminal investment. *Behav. Ecol. Sociobiol.* 71:185. doi: 10.1007/s00265-017-2416-z

Dugatkin, L. A., and Godin, J.-G. J. (1992). Prey approaching predators: a cost-benefit perspective. *Ann. Zool. Fennici* 29, 233–252.

Duncan, C. J., and Sheppard, P. M. (1965). Sensory discrimination and its role in the evolution of Batesian mimicry. *Behaviour* 24, 269–282.

Dunsmoor, J. E., Bandettini, P. A., and Knight, D. C. (2007). Impact of continuous versus intermittent CS-UCS pairing on human brain activation during Pavlovian fear conditioning. *Behav. Neurosci.* 121, 635–642. doi: 10.1037/0735-7044.121.4.635

Eason, C. T., Murphy, E. C., Wright, G. R. G., and Spurr, E. B. (2002). Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. *Ecotoxicology* 11, 35–48. doi: 10.1023/A:1013793029831

Eisenberg, J. F., and Kleiman, D. G. (1972). Olfactory communication in mammals. *Annu. Rev. Ecol. Syst.* 3, 1–32.

Elliott, J. E., Hindmarch, S., Albert, C. A., Emery, J., Mineau, P., and Maisonneuve, F. (2014). Exposure pathways of anticoagulant rodenticides to nontarget wildlife. *Environ. Monit. Assess.* 186, 895–906. doi: 10.1007/s10661-013-3422-x

Embar, K., Kotler, B. P., and Mukherjee, S. (2011). Risk management in optimal foragers: the effect of sightlines and predator type on patch use, time allocation, and vigilance in gerbils. *Oikos* 120, 1657–1666. doi: 10.1111/j.1600-0706.2011.19278.x

Emile, N., and Barros, M. (2009). Recognition of a 3D snake model and its 2D photographic image by captive black tufted-ear marmosets (*Callithrix penicillata*). *Anim. Cogn.* 12, 725–732. doi: 10.1007/s10071-009-0234-z

Fancourt, B. A., and Jackson, R. B. (2014). Regional seroprevalence of *Toxoplasma gondii* antibodies in feral and stray cats (*Felis catus*) from Tasmania. *Aust. J. Zool.* 62, 272–283. doi: 10.1071/ZO14015

Fancourt, B. A., Nicol, S. C., Hawkins, C. E., Jones, M. E., and Johnson, C. N. (2014). Beyond the disease: Is *Toxoplasma gondii* infection causing population declines in the eastern quoll (*Dasyurus viverrinus*)? *Int. J. Parasitol. Parasites Wildl.* 3, 102–112. doi: 10.1016/j.ijppaw.2014.05.001

Fanson, B. G., Fanson, K. V., and Brown, J. S. (2010). Ecological factors affecting the foraging behaviour of *Xerus rutilus*. *African Zool.* 45, 265–272. doi: 10.3377/004.045.0205.

Ferrero, D. M., Lemon, J. K., Fluegge, D., Pashkovski, S. L., Korzan, W. J., Datta, S. R., et al. (2011). Detection and avoidance of a carnivore odor by prey. *Proc. Natl. Acad. Sci. U.S.A.* 108, 11235–11240. doi: 10.1073/pnas.1103317108

Fishman, M. A. (1999). Predator inspection: closer approach as a way to improve assessment of potential threats. *J. Theor. Biol.* 196, 225–235.

FitzGibbon, C. D., and Fanshawe, J. H. (1988). Stotting in Thomson's gazelles: an honest signal of condition. *Behav. Ecol. Sociobiol.* 23, 69–74.

Food and Agriculture Organization of the United Nations (2018). *AGP - Integrated Pest Management*.

Fortin, D., Boyce, M. S., Merrill, E. H., and Fryxell, J. M. (2004). Foraging costs of vigilance in large mammalian herbivores. *Oikos* 107, 172–180. doi: 10.1111/j.0030-1299.2004.12976.x

Fraenkel, G. S. (1959). The raison d'etre of secondary plant substances: these odd chemicals arose as a means of protecting plants from insects and now guide insects to food. *Science*129, 1466–1470.

Fraser, D. F., and Huntingford, F. A. (1986). Feeding and avoiding predation hazard: the behavioral response of the prey. *Ethology* 73, 56–68.

Freeland, W. J. (1991). "Plant secondary metabolites: biochemical coevolution with herbivores," in *Plant Defenses Against Mammalian Herbivory*, eds R. T. Palo and C. T. Robbins (Boca Raton, FL: CRC Press), 61–81.

Fuelling, O., and Halle, S. (2004). Breeding suppression in free-ranging grey-sided voles under the influence of predator odour. *Oecologia* 138, 151–159. doi: 10.1007/s00442-003-1417-y

Gabriel, M. W., Woods, L. W., Poppenga, R., Sweitzer, R. A., Thompson, C., Matthews, S. M., et al. (2012). Anticoagulant rodenticides on our public and community lands: spatial distribution of exposure and poisoning of a rare forest carnivore. *PLoS ONE* 7:e40163. doi: 10.1371/journal.pone.0040163

Garb, J., Kotler, B. P., and Brown, J. S. (2000). Foraging and community consequences of seed size for coexisting Negev desert granivores. *Oikos* 88, 291–300. doi: 10.1034/j.1600-0706.2000.880207.x

Garvey, P. M., Glen, A. S., Clout, M. N., Wyse, S. V., Nichols, M., and Pech, R. P. (2017). Exploiting interspecific olfactory communication to monitor predators. *Ecol. Appl.* 27, 389–402. doi: 10.1002/eap.1483

Geddes, A. M. W. (1992). *The Relative Importance of Pre-Harvest Crop Pests in Indonesia*. Kent: NRI.

Glimcher, P. W. (2010). *Foundations of Neuroeconomic Analysis*. New York, NY: Oxford University Press. doi: 10.1093/acprof:oso/9780199744251.001.0001

Gonzalez, L. E., Rojnik, B., Urrea, F., Urdaneta, H., Petrosino, P., Colasante, C., et al. (2007). *Toxoplasma gondii* infection lower anxiety as measured in the plus-maze and social interaction tests in rats: a behavioral analysis. *Behav. Brain Res.* 177, 70–79.

Göth, A. (2001). Innate predator-recognition in Australian brush-turkey (*Alectura lathami*, Megapodiidae) hatchlings. *Behaviour* 138, 117–136. doi: 10.1163/156853901750077826

Götz, T., and Janik, V. M. (2011). Repeated elicitation of the acoustic startle reflex leads to sensitisation in subsequent avoidance behaviour and induces fear conditioning. *BMC Neurosci.* 12:30. doi: 10.1186/1471-2202-12-30

Götz, T., and Janik, V. M. (2015). Target-specific acoustic predator deterrence in the marine environment. *Anim. Conserv.* 18, 102–111. doi: 10.1111/acv.12141

Gould, L., and Sauther, M. L. (2007). "Anti-predator strategies in a diurnal prosimian, the ring-tailed lemur (*Lemur catta*), at the Beza Mahafaly special reserve, Madagascar," in *Primate Anti-Predator Strategies*, eds S. L. Gursky and K. A. I. Nekaris (Boston, MA: Springer), 275–288.

Griffin, A. S., Blumstein, D. T., and Evans, C. S. (2001). Training captive-bred or translocated animals to avoid predators. *Conserv. Biol.* 14, 1317–1326. doi: 10.1046/j.1523-1739.2000.99326.x

Griffin, A. S., and Evans, C. S. (2003). Social learning of antipredator behaviour in a marsupial. *Anim. Behav.* 66, 485–492. doi: 10.1006/anbe.2003.2207

Haapakoski, M., Hardenbol, A. A., and Matson, K. D. (2018). Exposure to chemical cues from predator-exposed conspecifics increases reproduction in a wild rodent. *Sci. Rep.* 8:17214. doi: 10.1038/s41598-018-35568-0

Hamilton, W. D. (1971). Geometry for the selfish herd. *J. Theor. Biol.* 31, 295–311.

Hansen, I., and Bakken, M. (1999). Livestock-guarding dogs in Norway: Part I. Interactions. *J. Range Manag.* 52, 2–6.

Hanson, M. T., and Coss, R. G. (1997). Age differences in the response of California ground Squirrels (*Spermophilus beecheyi*) to avian and mammalian predators. *J Comp Psychol.* 111, 174–184. doi: 10.1037/0735-7036.111.2.174

Hari Dass, S. A., and Vyas, A. (2014). *Toxoplasma gondii* infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. *Mol. Ecol.* 23, 6114–6122. doi: 10.1111/mec.12888

Harvell, C. D., and Tollrian, R. (1999). "Why inducible defenses," in *The Ecology and Evolution of Inducible Defenses* (Princeton, NJ: Princeton University Press), 3–9.

Hedrich, H. J., and Bullock, G. R. (2004). *The Laboratory Mouse*. London; San Diego, CA: Academic Press, Inc.

Heffner, H. E., and Heffner, R. S. (2007). Hearing ranges of laboratory animals. *J. Am. Assoc. Lab. Anim. Sci.* 46, 20–22. Available online at: http://docserver.ingentaconnect.com/deliver/connect/aalas/15596109/v46n1/s3.pdf?expires=1555201600&id=0000&titleid=72010024&checksum=BC36FE70988307B511EB26D083F16400

Heffner, H. E., and Heffner, R. S. (2008). High-frequency hearing. *Handb. Senses Audit.* 55:60. doi: 10.1016/B978-012370880-9.00004-9

Hegab, I. M., Shang, G., Ye, M., Jin, Y., Wang, A., Yin, B., et al. (2014). Defensive responses of Brandt's voles (*Lasiopodomys brandtii*) to chronic predatory stress. *Physiol. Behav.* 126, 1–7. doi: 10.1016/j.physbeh.2013.12.001

Herman, C. S., and Valone, T. J. (2000). The effect of mammalian predator scent on the foraging behavior of *Dipodomys merriami*. *Oikos* 91, 139–145. doi: 10.1034/j.1600-0706.2000.910113.x

Heyes, C. M. (1994). Social learning in animals: categories and mechanisms. *Biol. Rev.* 69, 207–231.

Hirsch, S. M., and Bolles, R. C. (1980). On the ability of prey to recognize predators. *Z. Tierpsychol.* 54, 71–84.

Hoffmann, F., Musolf, K., and Penn, D. J. (2009). Freezing urine reduces its efficacy for eliciting ultrasonic vocalizations from male mice. *Physiol. Behav.* 96, 602–605. doi: 10.1016/j.physbeh.2008.12.014

House, P. K., Vyas, A., and Sapolsky, R. (2011). Predator cat odors activate sexual arousal pathways in brains of *Toxoplasma gondii* infected rats. *PLoS ONE* 6:e23277. doi: 10.1371/journal.pone.0023277

Howald, G. R., Mineau, P., Elliott, J. E., and Cheng, K. M. (1999). Brodifacoum poisoning of avian scavengers during rat control on a seabird colony. *Ecotoxicology* 8, 431–447.

Huang, I. N., Krukar, J. D., and Miles, S. P. (1992). Effects of reinforcement schedules on rats' choice behavior in extinction. *J. Gen. Psychol.* 119, 201–211.

Hughes, A. (1979). A schematic eye for the rat. *Vision Res.* 19, 569–588.

Hughes, J. J., and Ward, D. (1993). Predation risk and distance to cover affect foraging behaviour in Namib desert gerbils. *Anim. Behav.* 46, 1243–1245. doi: 10.1006/anbe.1993.1320

Hurst, J. L., and Beynon, R. J. (2004). Scent wars: the chemobiology of competitive signalling in mice. *Bioessays* 26, 1288–1298. doi: 10.1002/bies.20147

Hutchison, W. M., Aitken, P. P., and Wells, W. P. (1980). Chronic *Toxoplasma* infections and familiarity-novelty discrimination in the mouse. *Ann. Trop. Med. Parasitol.* 74, 145–150.

Hutchison, W. M., Dunachie, J. F., Siim, J. C., and Work, K. (1969). Life cycle of *Toxoplasma gondii*. *Br. Med. J.* 4, 806–806.

Inglis, I. R. R., Shepherd, D. S. S., Smith, P., Haynes, P. J. J., Bull, D. S., Cowan, D. P. D. P., et al. (1996). Foraging behaviour of wild rats (*Rattus norvegicus*) towards new foods and bait containers. *Appl. Anim. Behav. Sci.* 47, 175–190.

Iribarren, C., and Kotler, B. P. (2012). Patch use and vigilance behaviour by Nubian ibex: the role of the effectiveness of vigilance. *Evol. Ecol. Res.* 14, 223–234. Available online at: http://www.evolutionary-ecology.com/issues/v14/n02/hhar2705.pdf

Irving, P. W., and Magurran, A. E. (1997). Context-dependent fright reactions in captive european minnows: the importance of naturalness in laboratory experiments. *Anim. Behav.* 53, 1193–1201.

John, A. (2014). Rodent outbreaks and rice pre-harvest losses in Southeast Asia. *Food Secur.* 6, 249–260. doi: 10.1007/s12571-014-0338-4

Kaukeinen, D. E. (1994). "Rodent control in practice: householders, pest control operators and municipal authorities," in *Rodent Pests and Their Control*, eds A. P. Buckle and R. H. Smith (Wallingford: CAB International), 249–271.

Kavaliers, M., and Choleris, E. (2001). Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neurosci. Biobehav. Rev.* 25, 577–586. doi: 10.1016/S0149-7634(01)00042-2

Kavaliers, M., Choleris, E., and Colwell, D. D. (2001a). Brief exposure to female odors "emboldens" male mice by reducing predator-induced behavioral and hormonal responses. *Horm. Behav.* 40, 497–509. doi: 10.1006/hbeh.2001.1714

Kavaliers, M., Choleris, E., and Colwell, D. D. (2001b). Learning from others to cope with biting flies: social learning of fear-induced conditioned analgesia and active avoidance. *Behav. Neurosci.* 115, 661–674. doi: 10.1037/0735-7044.115.3.661

Kelley, J. L., and Magurran, A. E. (2003). Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behav. Ecol. Sociobiol.* 54, 225–232. doi: 10.1007/s00265-003-0621-4

Kendig, M. D., Bowen, M. T., Kemp, A. H., and McGregor, I. S. (2011). Predatory threat induces huddling in adolescent rats and residual changes in early adulthood suggestive of increased resilience. *Behav. Brain Res.* 225, 405–414. doi: 10.1016/j.bbr.2011.07.058

Kerfoot, W. C., and Sih, A. (1987). *Predation: Direct and Indirect Impacts on Aquatic Communities*. Hanover, NH: University Press of New England.

Kikusui, T., Takigami, S., Takeuchi, Y., and Mori, Y. (2001). Alarm pheromone enhances stress-induced hyperthermia in rats. *Physiol. Behav.* 72, 45–50. doi: 10.1016/S0031-9384(00)00370-X

Kloppers, E. L., St. Clair, C. C., and Hurd, T. E. (2005). Predator-resembling aversive conditioning for managing habituated wildlife. *Ecol. Soc.* 10:31. doi: 10.5751/ES-01293-100131

Koehler, A. E., Marsh, R. E., and Salmon, T. P. (1990). "Frightening methods and devices/stimuli to prevent mammal damage-a review," in *Proceedings of the Fourteenth Vertebrate Pest Conference 1990* (Lincoln, OR).

Kotler, B. P., Brown, J. S., Bouskila, A., Mukherjee, S., and Goldberg, T. (2004). Foraging games between gerbils and their predators: seasonal changes in schedules of activity and aprehension. *Isr. J. Zool.* 50, 256–271. doi: 10.1560/K8D7-8KCX-BLAW-Y2K5

Kotler, B. P., Brown, J. S., Dall, S. R. X., Gresser, S., Ganey, D. T., and Bouskila, A. (2002). Foraging games between gerbils and their predators: temporal dynamics of resource depletion and apprehension in gerbils. *Evol. Ecol. Res.* 4, 495–518. Available online at: http://www.evolutionary-ecology.com/issues/v04n04/ddar1310.pdf

Kotler, B. P., Brown, J. S., Mukherjee, S., Berger-Tal, O., and Bouskila, A. (2010). Moonlight avoidance in gerbils reveals a sophisticated interplay among time allocation, vigilance and state-dependent foraging. *Proc. R. Soc. B Biol. Sci.* 277, 1469–1474. doi: 10.1098/rspb.2009.2036

Krettek, J. E., and Price, J. L. (1977). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *J. Comp. Neurol.* 172, 687–722.

Krijger, I. M., Belmain, S. R., Singleton, G. R., Groot Koerkamp, P. W., and Meerburg, B. G. (2017). The need to implement the landscape of fear within rodent pest management strategies. *Pest Manag. Sci. U.S.A.* 73, 2397–2402. doi: 10.1002/ps.4626

LaManna, J. A., and Martin, T. E. (2016). Costs of fear: behavioural and life-history responses to risk and their demographic consequences vary across species. *Ecol. Lett.* 19, 403–413. doi: 10.1111/ele.12573

Lambert, O., Pouliquen, H., Larhantec, M., Thorin, C., and L'Hostis, M. (2007). Exposure of raptors and waterbirds to anticoagulant rodenticides (Difenacoum, bromadiolone, coumatetralyl, coumafen, brodifacoum): epidemiological survey in Loire Atlantique (France). *Bull. Environ. Contam. Toxicol.* 79, 91–94. doi: 10.1007/s00128-007-9134-6

Laundré, J. W., Hernández, L., and Altendorf, K. B. (2001). Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, U.S.A. *Can. J. Zool.* 79, 1401–1409. doi: 10.1139/z01-094

Leaver, L. A., and Daly, M. (2003). Effect of predation risk on selectivity in heteromyid rodents. *Behav. Processes* 64, 71–75. doi: 10.1016/s0376-6357(03)00108-6

Legg, C. R., and Lambert, S. (1990). Distance estimation in the hooded rat: experimental evidence for the role of motion cues. *Behav. Brain Res.* 41, 11–20.

Leigh, J., and Chamberlain, M. J. (2008). "Effects of aversive conditioning on behavior of nuisance Louisiana black bears," in *Human-Wildlife Conflicts,* Vol. 2, 175–182. Available online at: http://www.jstor.org/stable/24875663

Leirs, H. (2003). "Management of rodents in crops: the Pied Piper and his orchestra," in *Rats, Mice and People: Rodent Biology and Management* (Canberra, ACT: Australian Centre for International Agricultural Research), 183–190.

Lima, L. L., and Salmon, T. P. (2010). "Assessing some potential environmental impacts from agricultural anticoagulant uses," in *Proceedings os the 24th Vertebrate Pest Conference*, eds R. M. Timm and K. A. Fagerstone (Davis, CA: University of California), 199–203.

Lima, S. L. (1990). Evolutionarily stable antipredator behavior among isolated foragers: on the consequences of successful escape. *J. Theor. Biol.* 143, 77–89.

Lima, S. L. (1998). "Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives," in *Advances in the Study of Behavior*, eds A. P. Møller, M. Milinski, and P. J. Slater (Academic Press), 215–290.

Lima, S. L., and Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am. Nat.* 153, 649–659.

Lima, S. L., and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619–640.

Litvin, Y., Blanchard, D. C., and Blanchard, R. J. (2007). Rat 22kHz ultrasonic vocalizations as alarm cries. *Behav. Brain Res.* 182, 166–172. doi: 10.1016/j.bbr.2006.11.038

Lohr, M. T., and Davis, R. A. (2018). Anticoagulant rodenticide use, non-target impacts and regulation: a case study from Australia. *Sci. Total Environ.* 634, 1372–1384. doi: 10.1016/j.scitotenv.2018.04.069

Lore, R., Blanc, A., and Suedfeld, P. (1971). Empathic learning of a passive-avoidance response in domesticated *Rattus norvegicus*. *Anim. Behav.* 19, 112–114.

Lund, M. (2015). "Commensal rodents," in *Rodent Pests and Their Control*, eds A. Buckle and R. H. Smith (Wallingford: CAB International), 19–32.

Lyly, M. S., Koivisto, E., Huitu, O., and Korpimäki, E. (2018). Simulated owl predation risk to voles modifies browsing effects on tree seedling growth. *Ann. Zool. Fennici* 55, 93–101. doi: 10.5735/086.055.0109

Ma, M. (2012). "Odor and pheromone sensing via chemoreceptors," in *Sensing in nature*, ed C. López-Larrea (New York, NY: Springer), 93–106.

Macdonald, D. W., Mathews, F., and Berdoy, M. (1999). "The behaviour and ecology of *Rattus norvegicus*: from opportunism to kamikaze tendencies," in *Ecologically-Based Rodent Management*, eds G. R. Singleton, L. A. Hinds, H. Leirs, and Z. Zhang (Canberra, ACT: Australian Centre for International Agricultural Research), 49–80.

MacLeod, K. J., Krebs, C. J., Boonstra, R., and Sheriff, M. J. (2018). Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. *Oikos* 127, 375–380. doi: 10.1111/oik.04890

Magurran, A. E. (1989). Acquired recognition of predator odour in the European Minnow (*Phoxinus phoxinus*). *Ethology* 82, 216–223.

Magurran, A. E., and Girling, S. L. (1986). Predator model recognition and response habituation in shoaling minnows. *Anim. Behav.* 34, 510–518.

Mahlaba, T. A., Monadjem, A., McCleery, R., and Belmain, S. R. (2017). Domestic cats and dogs create a landscape of fear for pest rodents around rural homesteads. *PLoS ONE* 12:e0171593. doi: 10.1371/journal.pone.0171593

Makundi, R. H., Oguge, N. O., and Mwanjabe, P. S. (1999). "Rodent pest management in East Africa - an ecological approach," in *Ecologically-Based*

Rodent Management, eds G. R. Singleton, L. A. Hinds, H. Leirs, and Z. Zhang (Canberra, ACT: Australian Centre for International Agricultural Research), 460–476.

Mao, J. S., Boyce, M. S., Smith, D. W., Singer, F. J., Vales, D. J., Vore, J. M., et al. (2010). Habitat selection by elk before and after wolf reintroduction in Yellowstone National Park. *J. Wildl. Manage.* 69, 1691–1707. doi: 10.2193/0022-541X(2005)69[1691:HSBEBA]2.0.CO;2

Martin, T. E. (2011). The cost of fear. *Science* 334, 1353–1354. doi: 10.1126/science.1216109

Martínez, A. E., Parra, E., Collado, L. F., and Vredenburg, V. T. (2017). Deconstructing the landscape of fear in stable multi-species societies. *Ecology* 98, 2447–2455. doi: 10.1002/ecy.1935

Matassa, C. M., and Trussell, G. C. (2011). Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. *Ecology* 92, 2258–2266. doi: 10.1890/11-0424.1

Mathis, A., and Vincent, F. (2000). Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). *Can. J. Zool.* 78, 1646–1652. doi: 10.1139/z00-090

Mccleery, R. A. (2009). Changes in fox squirrel anti-predator behaviors across the urban–rural gradient. *Landsc. Ecol.* 24:483. doi: 10.1007/s10980-009-9323-2

McGregor, I. S., Hargreaves, G. A., Apfelbach, R., and Hunt, G. E. (2004). Neural correlates of cat odor-induced anxiety in rats: region-specific effects of the benzodiazepine midazolam. *J. Neurosci.* 24, 4134–4144. doi: 10.1523/jneurosci.0187-04.2004

McNally, G. P., Johansen, J. P., and Blair, H. T. (2011). Placing prediction into the fear circuit. *Trends Neurosci.* 34, 283–292. doi: 10.1016/j.tins.2011.03.005

Meehan, A. P. (1984). *Rats and Mice. Their Biology and Control*. East Grinstead: Rentokil Ltd.

Meerburg, B. G., Singleton, G. R., and Kijlstra, A. (2009a). Rodent-borne diseases and their risks for public health. *Crit. Rev. Microbiol.* 35, 221–270. doi: 10.1080/10408410902989837

Meerburg, B. G., Singleton, G. R., and Leirs, H. (2009b). The year of the rat ends—time to fight hunger! *Pest Manag. Sci.* 65, 351–352. doi: 10.1002/ps.1718

Meerburg, B. G., van Gent-Pelzer, M. P., Schoelitsz, B., and van der Lee, T. A. (2014). Distribution of anticoagulant rodenticide resistance in *Rattus norvegicus* in the Netherlands according to Vkorc1 mutations. *Pest Manag. Sci.* 70, 1761–1766. doi: 10.1002/ps.3809

Miller, M. W., and Vogt, B. A. (1984). Direct connections of rat visual cortex with sensory, motor, and association cortices. *J. Comp. Neurol.* 226, 184–202.

Mills, J. N. (1999). "The role of rodents in emerging human disease: examples from the hantaviruses and arenaviruses," in *Ecologically-Based Rodent Management*, eds G. R. Singleton, L. A. Hinds, H. Leirs, and Z. Zhang (Canberra, ACT: Australian Centre for International Agricultural Research), 134–160.

Mineka, S., and Cook, M. (1988). "Social learning and the acquisition of snake fear in monkeys," in *Social Learning: Psychological and Biological Perspectives*, eds T. R. Zentall, J. B. G. Galef, and T. R. Zentall (Mahwah, NJ: Lawrence Erlbaum Associates, Inc.), 24.

Mirza, R. S., and Chivers, D. P. (2000). Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Can. J. Zool.* 78, 2198–2208. doi: 10.1139/z00-164

Mitra, R., Sapolsky, R. M., and Vyas, A. (2012). *Toxoplasma gondii* infection induces dendritic retraction in basolateral amygdala accompanied by reduced corticosterone secretion. *Dis. Model. Mech.* 6, 516–520. doi: 10.1242/dmm.009928

Monclús, R., Rödel, H. G., Von Holst, D., and De Miguel, J. (2005). Behavioural and physiological responses of naïve European rabbits to predator odour. *Anim. Behav.* 70, 753–761. doi: 10.1016/j.anbehav.2004.12.019

Mongeau, R., Miller, G. A., Chiang, E., and Anderson, D. J. (2003). Neural correlates of competing fear behaviors evoked by an innately aversive stimulus. *J. Neurosci.* 23, 3855–3868. doi: 10.1523/JNEUROSCI.23-09-03855.2003

Morris, D. W. (1997). Optimally foraging deer mice in prairie mosaics: a test of habitat theory and absence of landscape effects. *Oikos* 80, 31–42.

Mucignat-Caretta, C. (2010). The rodent accessory olfactory system. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* 196, 767–777. doi: 10.1007/s00359-010-0555-z

Muller-Schwarze, D. (2006). *Chemical Ecology of Vertebrates*. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511607233

Naidenko, S. V., Naidenko, S. V., Clark, L., and Voznessenskaya, V. V. (2003). *Predator Presence Affects the Reproductive Success of Prey in Outdoor Conditions*. Vol. 203, 1–6. Available online at: https://digitalcommons.unl.edu/icwdm_usdanwrc/203

National Research Council (2010). *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academies Press.

Nowak, K., Le Roux, A., Richards, S. A., Scheijen, C. P. J., and Hill, R. A. (2014). Human observers impact habituated samango monkeys' perceived landscape of fear. *Behav. Ecol.* 25, 1199–1204. doi: 10.1093/beheco/aru110

Ojeda, F. P., and Muñoz, A. A. (1999). Feeding selectivity of the herbivorous fish *Scartichthys viridis*: effects on macroalgal community structure in a temperate a rocky intertidal coastal zone. *Mar. Ecol. Prog. Ser.* 184, 219–229.

Osada, K., Miyazono, S., and Kashiwayanagi, M. (2014). Pyrazine analogs are active components of wolf urine that induce avoidance and fear-related behaviors in deer. *Front. Behav. Neurosci.* 8:276. doi: 10.3389/fnbeh.2014.00276

Owens, N. W. (1977). Responses of wintering brent geese to human disturbance. *Wildfowl* 28, 5–14.

Pangle, K. L., Peacor, S. D., and Johannsson, O. E. (2007). Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology* 88, 402–412. doi: 10.1890/06-0768

Parsons, M. H., Apfelbach, R., Banks, P. B., Cameron, E. Z., Dickman, C. R., Frank, A. S. K., et al. (2017). Biologically meaningful scents: a framework for understanding predator-prey research across disciplines. *Biol. Rev.* 93, 98–114. doi: 10.1111/brv.12334

Parsons, M. H., and Blumstein, D. T. (2010). Familiarity breeds contempt: kangaroos persistently avoid areas with experimentally deployed dingo scents. *PLoS ONE* 5:e10403. doi: 10.1371/journal.pone.0010403

Pelz, H.-J. (2007). Spread of resistance to anticoagulant rodenticides in Germany. *Int. J. Pest Manag.* 53, 299–302. doi: 10.1080/09670870701245223

Pelz, H. J., Rost, S., Hünerberg, M., Fregin, A., Heiberg, A. C., Baert, K., et al. (2005). The genetic basis of resistance to anticoagulants in rodents. *Genetics* 170, 1839–1847. doi: 10.1534/genetics.104.040360

Pérez-Gómez, A., Bleymehl, K., Stein, B., Pyrski, M., Birnbaumer, L., Munger, S. D., et al. (2015). Innate predator odor aversion driven by parallel olfactory subsystems that converge in the ventromedial hypothalamus. *Curr. Biol.* 25, 1340–1346. doi: 10.1016/j.cub.2015.03.026

Perrot-Sinal, T., Ossenkopp, K. P., and Kavaliers, M. (2000). Influence of a natural stressor (predator odor) on locomotor activity in the meadow vole (*Microtus pennsylvanicus*): modulation by sex, reproductive condition and gonadal hormones. *Psychoneuroendocrinology* 25, 259–276. doi: 10.1016/S0306-4530(99)00054-2

Perrot-Sinal, T. S., Heale, V. R., Ossenkopp, K. -P., and Kavaliers, M. (1996). Sexually dimorphic aspects of spontaneous activity in meadow voles (*Microtus pennsylvanicus*): effects of exposure to fox odor. *Behav. Neurosci.* 110, 1126–1132. doi: 10.1037/0735-7044.110.5.1126

Perrot-Sinal, T. S., Ossenkopp, K.-P. P., and Kavaliers, M. (1999). Effects of repeated exposure to fox odor on locomotor activity levels and spatial movement patterns in breeding male and female meadow voles (*Microtus pennsylvanicus*). *J. Chem. Ecol.* 25, 1567–1584.

Piekarski, G., Zippelius, H. M., and Witting, P. A. (1978). Auswirkungen einer latenten *Toxoplasma*-infektion auf das lernvermogen von weiben laboratoriumsratten und -mausen. *Z. Parasitenkd.* 57, 1–15.

Portfors, C. V (2007). Types and functions of ultrasonic vocalizations in laboratory rats and mice. *J. Am. Assoc. Lab. Anim. Sci.* 46, 28–34. Available online at: http://docserver.ingentaconnect.com/deliver/connect/aalas/15596109/v46n1/s5.pdf?expires=1555202270&id=0000&titleid=72010024&checksum=5B489695FC2D58757E685F6847D2A840

Pulliam, H. R. (1973). On the advantages of flocking. *J. Theor. Biol.* 38, 419–422.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., et al. (2009). Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol. Learn. Mem.* 92, 135–138. doi: 10.1016/j.nlm.2008.09.012

Rasmussen, L. E. (1988). Chemosensory responses in two species of elephants to constituents of temporal gland secretion and musth urine. *J. Chem. Ecol.* 14, 1687–1711. doi: 10.1007/BF01014552

Rattner, B. A., and Mastrota, F. N. (2018). "Anticoagulant rodenticide toxicity to non-target wildlife under controlled exposure conditions," in *Anticoagulant Rodenticides and Wildlife*, eds N. W. van den Brink, J. E. Elliott, R. F. Shore, and B. A. Rattner (Cham: Springer International Publishing), 45–86.

Raveh, A., Kotler, B. P., Abramsky, Z., and Krasnov, B. R. (2011). Driven to distraction: detecting the hidden costs of flea parasitism through foraging behaviour in gerbils. *Ecol. Lett.* 14, 47–51. doi: 10.1111/j.1461-0248.2010.01549.x

Remtulla, S., and Hallett, P. E. (1985). A schematic eye for the mouse, and comparisons with the rat. *Vision Res.* 25, 21–31.

Rescorla, R. A., and Wagner, A. R. (1972). "A theory of Pavlovian conditioning : variations in the effectiveness of reinforcement and nonreinforcement," in *Classical Conditioning II: Current Research and Theory*, eds A. H. Black and W. F. Prokasy (New York, NY: Appleton-Century-Crofts), 64–99.

Rodriguez, M. J. A. (1993). *Roedores Plagas: un Problema Permanente en America Latina y el Caribe*. Santiago: Oficina Regional de la FAO para America Latina y el Caribe.

Rost, S., Pelz, H. -J., Menzel, S., MacNicoll, A. D., León, V., Song, K. -J., et al. (2009). Novel mutations in the VKORC1 gene of wild rats and mice – a response to 50 years of selection pressure by warfarin? *BMC Genet.* 10:4. doi: 10.1186/1471-2156-10-4

Rusiniak, K. W., Hankins, W. G., Garcia, J., and Brett, L. P. (1979). Flavor-illness aversions: potentiation of odor by taste in rats. *Behav. Neural Biol.* 25, 1–17.

Russell, J. T. (1932). Depth discrimination in the rat. *Pedagog. Semin. J. Genet. Psychol.* 40, 136–161.

Russon, A. E. (1997). "Exploiting the expertise of others," in *Machiavellian Intelligence II: Extensions and Evaluations*, eds A. Whiten and R. W. Byrne (Cambridge: Cambridge University Press), 174–206.

Ruxton, G. D., and Lima, S. L. (1997). Predator–induced breeding suppression and its consequences for predator–prey population dynamics. *Proc. R. Soc. London. Ser. B Biol. Sci.* 264, 409–415.

Samia, D. S., Nakagawa, S., Nomura, F., Rangel, T. F., and Blumstein, D. T. (2015). Increased tolerance to humans among disturbed wildlife. *Nat. Commun.* 6:8877. doi: 10.1038/ncomms9877

Sanders, J., Mayford, M., and Jeste, D. (2013). Empathic fear responses in mice are triggered by recognition of a shared experience. *PLoS ONE* 8:e74609. doi: 10.1371/journal.pone.0074609

Schakner, Z. A., and Blumstein, D. T. (2013). Behavioral biology of marine mammal deterrents: a review and prospectus. *Biol. Conserv.* 167, 380–389. doi: 10.1016/j.biocon.2013.08.024

Schaller, G. B. (1972). *The Serengeti Lion: A Study of Predator-Prey Relations*. Chicago, IL: University of Chicago Press.

Scherer, A. E., and Smee, D. L. (2016). A review of predator diet effects on prey defensive responses. *Chemoecology* 26, 83–100. doi: 10.1007/s00049-016-0208-y

Schmeisser, E., Pollard, K. A., and Letowski, T. (2013). *Olfaction Warfare: Odor as Sword and Shield*. Aberdeen: Army Research Laboratory. doi: 10.21236/ADA577342

Schmidt, K. A., Brown, J. S., and Morgan, R. A. (1998). Plant defenses as complementary resources: a test with squirrels. *Oikos* 81, 130–142.

Schmitz, O. J., Beckerman, A. P., and O'Brien, K. M. (1997). Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology* 78, 1388–1399.

Schoeppner, N. M., and Relyea, R. A. (2009). Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Funct. Ecol.* 23, 1114–1121. doi: 10.1111/j.1365-2435.2009.01578.x

Schulte, B. A. (2016). "Learning and applications of chemical signals in vertebrates for human–wildlife conflict mitigation," in *Chemical Signals in Vertebrates 13*, eds B. A. Schulte, T. E. Goodwin, and M. H. Ferkin (Cham: Springer International Publishing), 499–510.

Schwanz, L. E., Brisson, D., Gomes-Solecki, M., and Ostfeld, R. S. (2011). Linking disease and community ecology through behavioural indicators: immunochallenge of white-footed mice and its ecological impacts. *J. Anim. Ecol.* 80, 204–214. doi: 10.1111/j.1365-2656.2010.01745.x

Schwanz, L. E., Previtali, M. A., Gomes-Solecki, M., Brisson, D., and Ostfeld, R. S. (2012). Immunochallenge reduces risk sensitivity during foraging in white-footed mice. *Anim. Behav.* 83, 155–161. doi: 10.1016/j.anbehav.2011.10.020

Semlitsch, R. D., and Reyer, H.-U. (1992). Modification of anti-predator behaviour in tadpoles by environmental conditioning. *J. Anim. Ecol.* 61, 353–360.

Shoham, S., Marcus, E. -L., Avraham, Y., and Berry, E. M. (2000). Diet restriction increases enkephalin- and dynorphin-like immunoreactivity in rat brain and attenuates long-term retention of passive avoidance. *Nutr. Neurosci.* 3, 41–55. doi: 10.1080/1028415X.2000.11747302

Siegmund, A., and Wotjak, C. T. (2007). A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. *J. Psychiatr. Res.* 41, 848–860. doi: 10.1016/j.jpsychires.2006.07.017

Singleton, G. R. (2003). *Impacts of Rodents on Rice Production in Asia*. Los Baños: International Rice Research Institute. Available online at: http://books.irri.org/DPS45_content.pdf

Singleton, G. R., Brown, P. R., Jacob, J., and Aplin, K. P. (2007). Unwanted and unintended effects of culling: a case for ecologically-based rodent management. *Integr. Zool.* 2, 247–259. doi: 10.1111/j.1749-4877.2007.00067.x

Singleton, G. R., Hinds, L. A., Leirs, H., and Zhang, Z. (1999). *Ecologically-Based Rodent Management*, eds G. R. Singleton, L. A. Hinds, H. Leirs, and Z. Zhang (Canberra, ACT: Australian Centre for International Agricultural Research).

Smith, J. A., Suraci, J. P., Clinchy, M., Crawford, A., Roberts, D., Zanette, L. Y., et al. (2017). Fear of the human 'super predator' reduces feeding time in large carnivores. *Proc. R. Soc. B Biol. Sci.* 284:20170433. doi: 10.1098/rspb.2017.0433

Sparrow, E. E., Parsons, M. H., and Blumstein, D. T. (2016). Novel use for a predator scent: preliminary data suggest that wombats avoid recolonising collapsed burrows following application of dingo scent. *Aust. J. Zool.* 64, 192–197 doi: 10.1071/ZO15068

Srivastava, A. (1991). Cultural transmission of snake-mobbing in free-ranging Hanuman langurs. *Folia Primatol.* 56, 117–120.

Staddon, J. E. R. (1993). On rate-sensitive habituation. *Adapt. Behav.* 1, 421–436.

Stankowich, T., and Blumstein, D. T. (2005). Fear in animals: a meta-analysis and review of risk assessment. *Proc. R. Soc. B Biol. Sci.* 272, 2627–2634. doi: 10.1098/rspb.2005.3251

Staples, L. G., Hunt, G. E., Cornish, J. L., and McGregor, I. S. (2005). Neural activation during cat odor-induced conditioned fear and 'trial 2' fear in rats. *Neurosci. Biobehav. Rev.* 29, 1265–1277. doi: 10.1016/j.neubiorev.2005.04.009

Staples, L. G., McGregor, I. S., Apfelbach, R., and Hunt, G. E. (2008). Cat odor, but not trimethylthiazoline (fox odor), activates accessory olfactory and defense-related brain regions in rats. *Neuroscience* 151, 937–947. doi: 10.1016/j.neuroscience.2007.11.039

Stenseth, N. C., Leirs, H., Skonhoft, A., Davis, S. A., Pech, R. P., Andreassen, H. P., et al. (2003). Mice, rats, and people: the bio-economics of agricultural rodent pests. *Front. Ecol. Environ.* 1, 367–375. doi: 10.1890/1540-9295(2003)001[0367:MRAPTB]2.0.CO;2

Stephens, D. W., and Krebs, J. (1986). *Foraging Theory*, *1st Edn*, eds J. R. Krebs and T. Clutto-Brock (Princeton, NJ: Princenton University Press).

Stephens, M. A., and Wand, G. (2012). Stress and the HPA axi: role of glucocorticoids in alcohol dependence. *Alcohol Res. Curr. Rev.* 34, 468–483. Available online at: https://pubs.niaaa.nih.gov/publications/arcr344/468-483.pdf

Suraci, J. P., Clinchy, M., Dill, L. M., Roberts, D., and Zanette, L. Y. (2016). Fear of large carnivores causes a trophic cascade. *Nat. Commun.* 7:10698. doi: 10.1038/ncomms10698

Suraci, J. P., Clinchy, M., Mugerwa, B., Delsey, M., Macdonald, D. W., Smith, J. A., et al. (2017a). A new automated behavioural response system to integrate playback experiments into camera trap studies. *Methods Ecol. Evol.* 8, 957–964. doi: 10.1111/2041-210X.12711

Suraci, J. P., Roberts, D. J., Clinchy, M., and Zanette, L. Y. (2017b). Fearlessness towards extirpated large carnivores may exacerbate the impacts of naïve mesocarnivores. *Behav. Ecol.* 28. doi: 10.1093/beheco/arw178

Swanepoel, L. H., Swanepoel, C. M., Brown, P. R., Eiseb, S. J., Goodman, S. M., Keith, M., et al. (2017). A systematic review of rodent pest research in Afro-Malagasy small-holder farming systems: are we asking the right questions? *PLoS ONE* 12:e0174554. doi: 10.1371/journal.pone.0174554

Talling, J. C., Waran, N. K., Wathes, C. M., and Lines, J. A. (1998). Sound avoidance by domestic pigs depends upon characteristics of the signal. *Appl. Anim. Behav. Sci.* 58, 255–266.

Taylor, K. D., Hammond, L. E., and Quy, R. J. (1974). The reactions of common rats to four types of live-capture trap. *J. Appl. Ecol.* 11, 453–459.

Taylor, R. J. (1984). *Predation*. New York, NY: Springer Netherlands doi: 10.1007/978-94-009-5554-7

Tenter, A. M., Heckeroth, A. R., and Weiss, L. M. (2000). *Toxoplasma gondii*: from animals to humans. *Int. J. Parasitol.* 30, 1217–1258. doi: 10.1016/S0020-7519(00)00124-7

Thijssen, H. H. W. (1995). Warfarin based rodenticides: mode of action and mechanism of resistance. *Pestic. Sci.* 43, 73–78.

Thomas, P. J., Mineau, P., Shore, R. F., Champoux, L., Martin, P. A., Wilson, L. K., et al. (2011). Second generation anticoagulant rodenticides in predatory birds: probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. *Environ. Int.* 37, 914–920. doi: 10.1016/j.envint.2011.03.010

Thorson, J. M., Morgan, R. A., Brown, J. S., and Norman, J. E. (1998). Direct and indirect cues of predatory risk and patch use by fox squirrels and thirteen-lined ground squirrels. *Behav. Ecol.* 9, 151–157.

Tobin, M. E., and Fall, M. W. (2006). "Pest control: rodents," in *U.S. Department of Agriculture's National Wildlife Research Center* (Staff Publication), 67, 1–21. Available online at: https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1065&context=icwdm_usdanwrc

Tollrian, R., and Harvell, C. D. (1999). "The evolution of inducible defenses: current ideas," in *The Ecology and Evolution of Inducible Defenses*, eds R. Tollrian and C. D. Harvell (Princeton, NJ: Princeton University Press), 306–321.

Topál, J., and Csányi, V. (1994). The effect of eye-like schema on shuttling activity of wild house mice (*Mus musculus domesticus*): context-dependent threatening aspects of the eyespot patterns. *Anim. Learn. Behav.* 22, 96–102.

Tromelin, A. (2016). Odour perception: a review of an intricate signalling pathway. *Flavour Fragr. J.* 31, 107–119. doi: 10.1002/ffj.3295

Turner, G. F., and Pitcher, T. J. (1986). Attack abatement: a model for group protection by combined avoidance and dilution. *Am. Nat.* 128, 228–240.

Uetz, G. W., and Hieber, C. S. (1994). Group size and predation risk in colonial web-building spiders: analysis of attack abatement mechanisms. *Behav. Ecol.* 5, 326–333.

Valeix, M., Loveridge, A. J., Chamaillé-Jammes, S., Davidson, Z., Murindagomo, F., Fritz, H., et al. (2009). Behavioral adjustments of African herbivores to predation risk by lions: spatiotemporal variations influence habitat use. *Ecology* 90, 23–30. doi: 10.1890/08-0606.1

van Donk, E., Lürling, M., and Lampert, W. (1999). "Consumer-induced changes in phytoplankton: inducibility, costs, benefits, and the impact on grazers," in *The Ecology and Evolution of Inducible Defenses*, eds R. Tollrian and C. D. Harvell (Princeton, NJ: Princeton University Press), 89–103.

van Eeden, L. M., Crowther, M. S., Dickman, C. R., Macdonald, D. W., Ripple, W. J., Ritchie, E. G., et al. (2017). Managing conflict between large carnivores and livestock. *Conserv. Biol.* 32, 26–34. doi: 10.1111/cobi.12959

Vasudevan, A., and Vyas, A. (2013). Kairomonal communication in mice is concentration-dependent with a proportional discrimination threshold. *F1000Research* 2, 195. doi: 10.12688/f1000research.2-195.v1

Veen, T., Richardson, D. S., Blaakmeer, K., and Komdeur, J. (2000). Experimental evidence for innate predator recognition in the Seychelles warbler. *Proc. R. Soc. London. Ser. B Biol. Sci.* 267, 2253–2258. doi: 10.1098/rspb.2000.1276

Verma, D., Wood, J., Lach, G., Herzog, H., Sperk, G., and Tasan, R. (2016). Hunger promotes fear extinction by activation of an amygdala microcircuit. *Neuropsychopharmacology* 41, 431–439. doi: 10.1038/npp.2015.163

Vernet-Maury, E., Polak, E. H., and Demael, A. (1984). Structure-activity relationship of stress-inducing odorants in the rat. *J. Chem. Ecol.* 10, 1007–1018.

Vilhunen, S., and Hirvonen, H. (2003). Innate antipredator responses of Arctic charr (*Salvelinus alpinus*) depend on predator species and their diet. *Behav. Ecol. Sociobiol.* 55, 1–10. doi: 10.1007/s00265-003-0670-8

Von Frisch, K. (1942). Über einen schreckstoff der fischhaut und seine biologische bedeutung. *Z. Vgl. Physiol.* 29, 46–145.

Voznessenskaya, V. V., Naidenko, S. V., Feoktistova, N. Y., Krivomazov, G. J., Miller, L. A., and Clark, L. (2003). *Predator Odours as Reproductive Inhibitors for Norway Rats*. USDA National Wildlife Research Center – Staff Publications, 251.

Vyas, A., Kim, S. K., Giacomini, N., Boothroyd, J. C., and Sapolsky, R. M. (2007b). Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc. Natl. Acad. Sci. U.S.A.* 104, 6442–6447. doi: 10.1073/pnas.0608310104

Vyas, A., Kim, S. K., and Sapolsky, R. M. (2007a). The effects of *Toxoplasma* infection on rodent behavior are dependent on dose of the stimulus. *Neuroscience* 148, 342–348. doi: 10.1016/j.neuroscience.2007.06.021

Vyas, A., and Sapolsky, R. (2010). Manipulation of host behaviour by *Toxoplasma gondii*: what is the minimum a proposed proximate mechanism should explain? *Folia Parasitol.* 57, 88–94. doi: 10.14411/fp.2010.011

Walker, L. A., Turk, A., Long, S. M., Wienburg, C. L., Best, J., and Shore, R. F. (2008). Second generation anticoagulant rodenticides in tawny owls (*Strix aluco*) from Great Britain. *Sci. Total Environ.* 392, 93–98. doi: 10.1016/j.scitotenv.2007.10.061

Wallace, D. J., Greenberg, D. S., Sawinski, J., Rulla, S., Notaro, G., and Kerr, J. N. (2013). Rats maintain an overhead binocular field at the expense of constant fusion. *Nature* 498, 65–69. doi: 10.1038/nature12153

Wang, L., Chen, I. Z., and Lin, D. (2015). Collateral pathways from the ventromedial hypothalamus mediate defensive behaviors. *Neuron* 85, 1344–1358. doi: 10.1016/j.neuron.2014.12.025

Ward, J. F., Macdonald, D. W., and Doncaster, C. P. (1997). Responses of foraging hedgehogs to badger odour. *Anim. Behav.* 53, 709–720.

Webster, J. P. (2007). The effect of *Toxoplasma gondii* on animal behavior: playing cat and mouse. *Schizophr. Bull.* 33, 752–756. doi: 10.1093/schbul/sbl073

Webster, J. P., Brunton, C. F., and Macdonald, D. W. (1994). Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* 109, 37–43. doi: 10.1017/S003118200007774X

Williams, J. L., Rogers, A. G., and Adler, A. P. (1990). Prolonged exposure to conspecific and predator odors reduces fear reactions to these odors during subsequent prod-shock tests. *Anim. Learn. Behav.* 18:453. doi: 10.3758/BF03205327

Wirsing, A. J., Heithaus, M. R., Frid, A., and Dill, L. M. (2008). Seascapes of fear: evaluating sublethal predator effects experienced and generated by marine mammals. *Mar. Mammal Sci.* 24, 1–15. doi: 10.1111/j.1748-7692.2007.00167.x

Witting, P. A. (1979). Learning capacity and memory of normal and *Toxoplasma*-infected laboratory rats and mice. *Zeitschrift für Parasitenkd.* 61, 29–51.

Yilmaz, M., and Meister, M. (2013). Rapid innate defensive responses of mice to looming visual stimuli. *Curr. Biol.* 23, 2011–2015. doi: 10.1016/j.cub.2013.08.015

Ylönen, H., and Brown, J. S. (2007). "Fear and the foraging, breeding, and sociality of rodents," in *Rodent Societies. An Ecological & Evolutionary Perspective*, eds J. O. Wolff and P. W. Sherman (Chicago, IL: The University of Chicago Press), 328–341.

Ylönen, H., Jacob, J., Davies, M. J., and Singleton, G. R. (2002). Predation risk and habitat selection of Australian house mice, *Mus domesticus*, during an incipient plague: desperate behaviour due to food depletion. *Oikos* 99, 284–289. doi: 10.1034/j.1600-0706.2002.990208.x

Zanette, L. Y., White, A. F., Allen, M. C., and Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* 334, 1398–1401. doi: 10.1126/science.1210908

Zoccolan, D., Oertelt, N., DiCarlo, J. J., and Cox, D. D. (2009). A rodent model for the study of invariant visual object recognition. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8748–8753. doi: 10.1073/pnas.0811583106

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Significant Genetic Impacts Accompany an Urban Rat Control Campaign in Salvador, Brazil

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Rats thrive in human-dominated landscapes and have expanded to a near global distribution. Norway rats (*Rattus norvegicus*) contaminate food, damage infrastructure, and are reservoirs for zoonotic pathogens that cause human diseases. To limit these negative impacts, entities around the world implement intervention and control strategies designed to quickly and drastically reduce the number of rats in a population. While the primary goal of these interventions is to reduce rat numbers and their detrimental activities, there are important, yet unexplored, population genetic implications for these rapid population declines. Here, we compare the population genetics of *R. norvegicus* before, immediately after, and several months following a rodenticide-based eradication campaign targeting rats in an urban slum of Salvador, Brazil. This slum has been the focus of long-term research designed to understand and reduce the risk of leptospirosis for people in this area. We also look for a clear source of rats contributing to population recovery, by either rebounding through breeding of local survivors, or by immigration/reinvasion of the site. We found evidence of severe genetic bottlenecks, with the effective population size dropping 85–91% after eradication, consistent with declines in population sizes. These rapid declines also led to a strong shift in the genetic structure of rats before and after the eradication campaign. Relatedness increased in two of the three study areas after eradication, suggesting reduced population sizes and an uneven impact of the campaign across colonies within the population. Lastly, dozens of low-frequency alleles (mean frequency of 0.037) observed before the campaign were undetected after the campaign, potentially lost from the population via drift or selection. We discuss the public health and ecological implications of these rapid genetic impacts of urban control efforts. Our data suggests that targeting the genetic viability of rat populations may be another important component for integrated pest management (IPM) strategies, designed to reduce urban rats.

Keywords: genetic bottleneck, eradication, invasive species, public health, vector control, intervention, integrated pest management, Rattus

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INTRODUCTION

The Norway rat, or brown rat (*Rattus norvegicus*), is a pest species that is invasive in much of its near-global range. It is responsible for billions of dollars in damage annually to properties, city infrastructure, and food stocks (Stenseth et al., 2003; Pimentel et al., 2005). *R. norvegicus* is also a vector and reservoir of many zoonotic pathogens, and is therefore an important species for public health monitoring and control (Easterbrook et al., 2007; Himsworth et al., 2013b; Firth et al., 2014). As a result of their impact on agricultural losses, human health, infrastructure, and native ecosystems, brown rats are one of the most important nuisance species globally (Capizzi et al., 2014; Parsons et al., 2017).

R. norvegicus has been the target of many intervention campaigns to reduce their numbers and the risk they pose to people and ecosystems. These campaigns are designed to reduce rat numbers, control their geographic spread, or completely remove all rats from an area. Efforts to completely eradicate rats have been limited mostly to islands separated geographically from other populations that may serve as sources of recolonizing rats (Howald et al., 2007; Russell et al., 2010; Savidge et al., 2012). However, cities across the world invest heavily to control or reduce rat populations in close proximity to dense human settlements, even if complete eradication is unlikely (Bonnefoy et al., 2008; de Masi et al., 2009; Parsons et al., 2017). Intense control efforts, through chemical rodenticide intervention and lethal trapping, are commonly implemented in cities around the world.

Despite the money and time spent reducing their numbers, rat population sizes usually rebound rapidly in urban areas after a control campaign, a pattern known as the "boomerang effect" (Smith, 1963; de Masi et al., 2009). The rats that repopulate a treated area may come from residual individuals that were not removed during the initial intervention. Hastening this internal rebound of rats is the fact that the initial campaign reduces competition for resources among the remaining rats, increasing reproductive rates and juvenile recruitment (Smith, 1963; Stokes et al., 2009; Vadell et al., 2010). Alternatively, rats may re-colonize the targeted area from nearby, untargeted areas. During equilibrium periods, rats from other colonies and areas may have trouble penetrating into or establishing in the range of other populations due to the tight social structure of rat colonies. The competitive advantage for rats in established populations/colonies are known as priority effects, making the invasion of new rats difficult (Fraser et al., 2015). However, a reduction in rat numbers during intervention campaigns can disrupt these priority effects, making re-colonization by rats from other areas more likely (Pichlmueller and Russell, 2018). Currently, it is not understood what role local residual vs. outside colonizing rats contribute to the inevitable rebound of rat population sizes in an urban context.

While the number of rats may consistently rebound after a control campaign, very little is known about the genetic impacts of a population decline and subsequent recovery in urban rats. Genetic variation and effective population size (N_e) are expected to decline along with census population size. Also, genetic variation generally takes much longer to replace in a population than the rate at which it is lost (Nei et al., 1975). Researchers have applied tests of heterozygosity excess to look for historic genetic bottleneck events in urban rats (Gardner-Santana et al., 2009; Desvars-Larrive et al., 2018). However, these tests for bottlenecks have not been in the context of a rodent-control campaign. Further, data comparing the genetics of pre-control samples to rats present after the control campaign are only available for island systems (Abdelkrim et al., 2007; Russell et al., 2010; Pichlmueller and Russell, 2018). For most eradication campaigns, the decline in rats happens within weeks, and the recovery within months. This makes the commonly used heterozygosity tests less useful since they operate on a time scale of 2–4 times the N_e of a population (Piry et al., 1999). Given that lag time, N_e can be compared directly between pre- and post-control samples to detect any recent loss of genetic variation. N_e represents the size of an "ideal" population that can be expected to lose genetic diversity (through genetic drift) at the same rate as the focal population, which often has a very different census population size (Frankham, 1995; Kalinowski and Waples, 2002; Husemann et al., 2016). N_e is also considered a much better indicator of the genetic status and evolutionary fitness of a population (Reed, 2005). In addition to genetic bottlenecks, rapid population declines can also result in shifts in the genetic structure of a population. Rats are a colonial species with close kin relationships with other rats in their colonies. Eradication campaigns likely disrupt these social structures (Clapperton, 2006), which have the potential to alter the relatedness levels of individuals repopulating an area post-intervention. Lethal control of rats has been linked to novel social interactions and transmission of pathogens (Himsworth et al., 2013a; Lee et al., 2018).

In this study, we took advantage of an intense eradication campaign that occurred in 2015 in Salvador, Brazil, a city of 2,900,000 people that has experienced a 500% increase in human population size in the last 60 years. Much of this increase has happened in favela (slum) settlements, with little sanitation infrastructure, high populations of rats, and elevated risk of zoonotic diseases for residents (Reis et al., 2008; Felzemburgh et al., 2014; Hagan et al., 2016). Here, we focus on Pau da Lima, a favela community targeted for rat eradication because it has experienced high levels of human leptospirosis infection (Ko et al., 1999; Reis et al., 2008). We sampled a total of 241 rats before ($n = 132$) and after ($n = 109$) the 2015 eradication campaign, genotyped them at 16 microsatellite loci, and conducted analyses to look for the degree of genetic bottlenecking that occurred. In addition, we analyzed if and how the population genetic structure shifted after the campaign, and if there was any signature of a disruption of colony systems through relatedness metrics. To our knowledge, this study represents the first investigation of genetic impacts of rodent control efforts in cities.

METHODS

Study Area and Sampling

We designed our sampling protocol around a rat-control intervention occurring within the Pau da Lima area of Salvador, Brazil in 2015. Pau da Lima is a favela with a high human population density and low socioeconomic status of residents, with 88% being considered squatters without a legal

right to residence (Reis et al., 2008). This neighborhood has been the focus of long-term epidemiological research trying to lower the risk of leptospirosis for human inhabitants (Costa et al., 2014; Felzemburgh et al., 2014). It is comprised of four connected valleys, and the samples in this study were collected from three of these valleys (Valley 1, 2, and 4; **Figure 1**); Valley 3 has not been part of the larger long-term eco-epidemiological study (Panti-May et al., 2016). Previous research has found genetic divisions between the valleys (Kajdacsi et al., 2013; Richardson et al., 2017), as well as multiple paternity and high relatedness among mating pairs (Costa et al., 2016).

We sampled 241 rats across the three Pau da Lima valleys, at three separate time points around a planned eradication campaign, using both live and snap traps (**Figure 1**). Between October and December 2014, we set traps at 108 sites across Pau da Lima and captured 34 rats from Valley 1, 47 rats from Valley 2, and 51 from Valley 4, representing 40 of the 108 trapping locations. The city of Salvador then conducted a lethal rat-control campaign between June and August of 2015. The intervention was performed by the Zoonotic Control Center (ZCC) of the Salvador city Secretary of Health. The ZCC's campaign included three pulses of rodenticide application as well as pre- and post-evaluation of rodent infestation, following visual-survey methodologies described previously in Brazil (de Masi et al., 2009) and other countries (Davis et al., 1977; Murphy and Oldbury, 2002). During a pre-campaign survey, the ZCC found that 39% of the households in the treatment area had signs of rodent infestation. This number dropped significantly to 21.1% ($p < 0.05$) after the campaign, indicating a 54% reduction in rat infestation in the evaluated households. We then trapped again 1 month after the eradication campaign ended, collecting 64 rats across the three valleys over a 3.5-week period between November and December of 2015 (**Figure 1**). Seven months after the campaign ended (March to April 2016), we collected tissue samples from another 45 rats trapped in the same areas of the three valleys (**Figure 1**). Trap sites at all time periods were geo-referenced and tail tissue was collected and stored in ethanol at −80°C until the DNA was extracted.

DNA Extraction and Genotyping

DNA was extracted from 2 to 5 mm tail tissue, using standard kit-based extraction protocols (Qiagen and ZyGEM). We then amplified 16 microsatellite loci previously identified as polymorphic in *Rattus spp.* using a touchdown PCR protocol (Kajdacsi et al., 2013). PCR amplicons were identified using capillary electrophoresis on an ABI 3730 DNA sequencer. GeneMarker software was used to score alleles and Microsatellite Toolkit v3.1 (Park, 2001) was used to check for scoring errors.

While loci were selected across chromosomes to prevent linkage disequilibrium, we evaluated whether loci conformed to Hardy-Weinberg equilibrium (HWE) for each locus in the R package "pegas" (Paradis, 2010), with significance estimated using 1,000 randomizations. Since the Pau da Lima valleys are unlikely to be a single gene pool, we separated each valley and year, and took the average HWE significance and tabulated the number of valley/year combinations. In doing so, only one locus (D5Cebr1) was identified as out of HWE and was therefore not used for subsequent analyses.

Genetic Bottleneck Analyses

We first looked for changes in the effective population size (N_e) in each valley before and after the eradication campaign. Even though rat populations rebound quickly after a control campaign, those census numbers do not reflect the genetic variation remaining in the population post-decline. The N_e is often much lower than the census population size for most species (Kalinowski and Waples, 2002; Husemann et al., 2016), averaging 10% of census size (Frankham, 1995). N_e is also a better indicator of the genetic viability and fitness of a population (Reed, 2005). In addition, our three sampling periods occurred over 16 months, or ∼3–6 rat generations. We used the change in N_e as our metric of genetic bottleneck levels because methods such as heterozygosity excess in the program BOTTLENECK, and *M*-ratio measures of allele number and size range variance detect bottleneck events over longer time spans ($2–4^*N_e$) than are relevant for our study (Piry et al., 1999; Garza and Williamson, 2001; Peery et al., 2012).

We estimated N_e using two approaches that utilize different properties of population genetics. We first estimated N_e using sibship assignment methods, based on the level of relatedness among individuals within a population (Wang, 2004, 2009). This analysis calculates sibship frequency (SF) levels, and was done in Colony2 using the recommended settings and a genotyping error rate of 0.01 (Jones and Wang, 2010). We also used NeEstimator (Do et al., 2014), which calculates N_e based on levels of linkage disequilibrium (LD). As N_e declines, genetic drift increases non-random associations among loci, thereby increasing signatures of LD (Hill, 1981; Waples, 2006). We used the random mating model and a threshold allele frequency of 0.02 (Waples and Do, 2008). Although both approaches require only a single sampling event, and have been shown to accurately estimate true N_e with sufficient sample sizes and polymorphic genetic loci (Wang, 2009; Waples and Do, 2010), the SF approach can be more accurate and robust to assumptions inherent to each estimate (Wang, 2016).

Discriminant Analysis of Genetic Structure

We used discriminant analysis of principal components (DAPC) to evaluate the overall genetic structure within the three Pau da Lima valleys, and if there were any changes in this structure after the eradication campaign. DAPC uses coefficients of locus allele loadings in linear combinations (PC axes) to maximize the variance between-groups while minimizing within-group variances in these loadings (Jombart et al., 2010). Empirical and simulated data have found that DAPC performs as well or better than other individual-based clustering methods [e.g., STRUCTURE; (Pritchard et al., 2000)], particularly when complex processes are operating (Jombart et al., 2010; Klaassen et al., 2012). In this analysis, we use DAPC to characterize basic genetic differences rather than to delineate new genetic groups. We used guidelines in the "*adegenet*" package v2.1.1 in R to conduct DAPC (Jombart et al., 2016). We retained the number of principal components necessary to explain ∼90% of the cumulative variance in each DAPC run, which ranged from 35

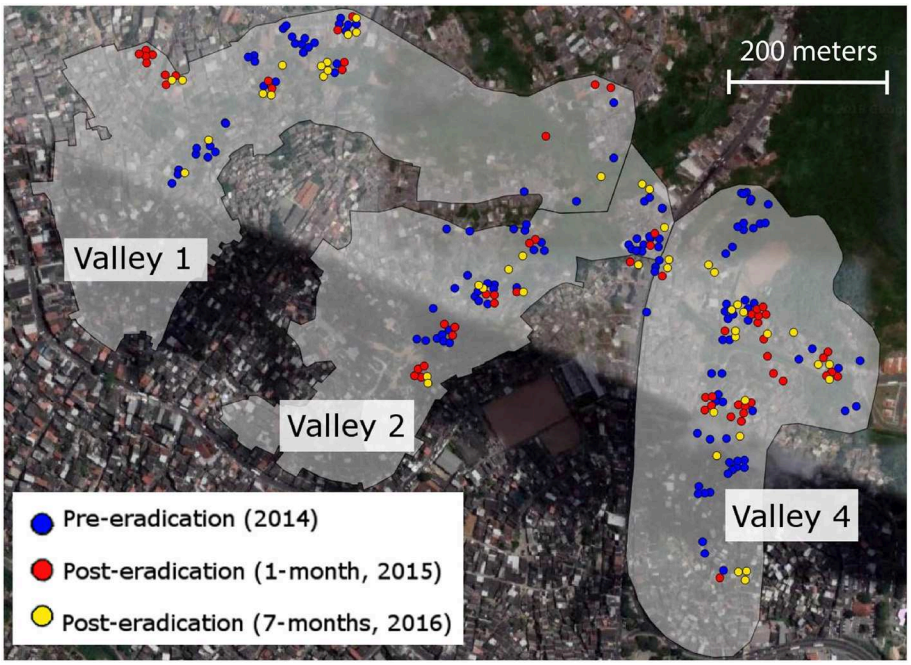

FIGURE 1 | The Pau da Lima section of Salvador, Brazil is comprised of four valleys, three of which were the target of an eradication campaign in mid-2015. We collected tissue samples from 241 rats trapped immediately before the campaign (blue points), and then 1-month (red points) and 7-months (gold points) after the campaign. These three valleys have been the focus of long-term epidemiological work to reduce human incidence of leptospirosis carried by *Rattus norvegicus* rats.

to 40 PCs. We also retained a single discriminant function for each run.

Relatedness

Norway rats exhibit colonial social systems, where individuals within a colony are more related to each other than at random among the population (Calhoun, 1963; Costa et al., 2016). However, drastic reductions in population size may disrupt this social structure, leading to shifts in relatedness if some colonies are hit harder than others, or if the residual individuals remaining after the campaign come from a subset of colonies and inbreeding increases (Calhoun, 1963). We estimated the level of relatedness for each pair of rats (i.e., dyad) in our dataset, within each time period sampled (i.e., pre-eradication, as well as 1- and 7-months post-eradication). We used the R package "*related*" to calculate both the Queller and Goodnight (Queller and Goodnight, 1989) and the dyad maximum likelihood (dyadML; Milligan, 2003) metrics of relatedness. Each is commonly used in the literature, with attendant benefits and caveats (Milligan, 2003; Goudet et al., 2018). We then used the R package *"dplyr"* to calculate summary statistics for the distribution of relatedness values in each valley and sampling period, and the packages *"ggplot2"* and *"ggpubr"* to plot the density distributions and regressions of both the Queller and dyadML metrics. Lastly, we performed a Kruskal-Wallis rank sum test in the base *"stats"* package of R, followed by pairwise Wilcoxon tests to determine which of the three distributions in each valley (i.e., which sampling time period) were significantly different from each of the other two.

Rare Allele Loss

During population bottlenecks and founder events, alleles are expected to be lost at a rate proportional to their frequency in the population (Maruyama and Fuerst, 1985). Through genetic drift, rare alleles are more likely to be lost than high frequency alleles, which concomitantly tend to increase in frequency during a bottleneck (Luikart et al., 1998). We calculated the frequency of every allele for each locus in each valley at each time period using the "readGenepop" function within the *"diversity"* package in R. We then identified the alleles that dropped out of the dataset between the pre- and post-eradication sampling periods and calculated the frequency of those alleles separately.

RESULTS

Genetic Bottleneck Analyses

There was a sharp drop in the effective population size (N_e) between the pre and post-eradication samples for each valley, with the sibship frequency (SF) estimation (**Figure 2A**). The N_e in Valley 1 decreased by 74% between the pre-eradication and 1-month post-eradication sampling period. The N_e in Valley 2 decreased by 65% and in Valley 4 by 42% during that same period (**Figure 2A**, blue vs. red bars). The N_e remained low or decreased further in the second post-eradication population, sampled 7 months following the campaign (**Figure 2A**, gold bars). N_e estimates based on linkage disequilibrium (LD) levels showed similar patterns, with Valley 1 and Valley 2 experiencing a 91% and 85% drop, respectively, in N_e after the eradication campaign (**Figure 2B**). N_e remained low during the samples

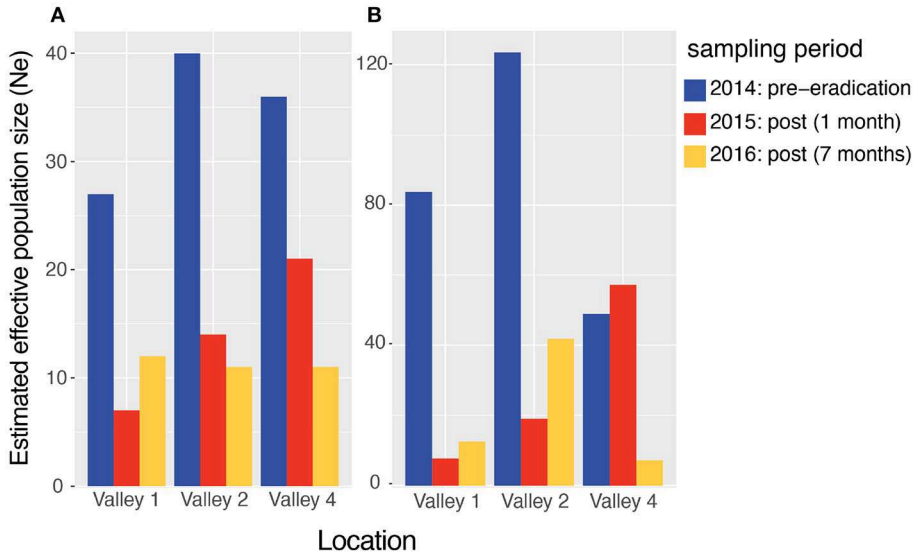

FIGURE 2 | Point estimates of effective population size (N_e) in three valleys of the Pau da Lima area of Salvador, Brazil. 241 rats were sampled just before the city conducted an eradication campaign to reduce the rat population (late-2014, blue bars), 1 month following the campaign (2015, red bars), and then 7-months later (early-2016, gold bars). Ne was estimated using two different methods: **(A)** the sibship frequency and **(B)** linkage disequilibrium approaches. Full data can be found in **Table S1**.

collected seven months after the campaign. Valley 4 did not show an immediate decline in LD-based N_e, as it increased by 16% between the first two sampling periods (**Figure 2B,** blue vs. red bars). However, consistent with all other valleys, there was an 85% decrease in N_e between the pre- and 7 months post-eradication sampling period (**Figure 2B**; gold bar).

To ensure that the N_e reductions were not an artifact of sample sizes that vary across the three sampling periods, we performed a rarefaction analysis and found that the results did not change qualitatively when we reduced the number of samples analyzed, to the minimum number collected at any time point. In fact, the reductions in N_e were larger in several cases. We also calculated N_e using combined genotype data from the two post-intervention sampling periods, but again, the results did not differ qualitatively. These data suggest that there are severe genetic consequences for urban rats that experience rapid population declines during lethal control initiatives.

Discriminant Analysis of Genetic Structure

There was a clear shift in the genetic structure of the rats in each valley before and after the eradication campaign (**Figure 3**). In each valley, the two post-eradication rat genotypes overlap significantly along the discriminant function axis. Post-eradication genotypes have minimal overlap with pre-eradication rat genotypes, suggesting that there was a rapid and intense change in the genetics of rat populations in each valley. The genotype discriminant distribution curve—a measure of genetic variation—showed less variation in Valley 1 than Valley 2 or Valley 4 (**Figure 3**). As with the N_e analysis above, we also ran a DAPC using combined genotype data from the two post-intervention sampling periods, in case the smaller sample sizes for each post-eradication period could shift results. But again, the results did not differ qualitatively from the default DAPCs done with each valley-time interval combination.

Relatedness

Valley 1 showed the highest levels of relatedness across all sampling periods, but the distributions of these values did not differ among the three time points sampled (**Figure 4**), suggesting no significant shift in the relatedness levels among rats in any sampling period. Rats from Valley 2 showed a significant increase in relatedness 1 month after the eradication campaign (**Figure 5**). This increase disappeared, though, 7 months after the campaign ended and the population rebounded (**Figure 5D**). Rats from Valley 4 also exhibited a significant increase in relatedness after the eradication campaign, which rose further 7 months after the campaign ceased (**Figure 6**). For all three valleys, the two measures of relatedness corresponded closely to each other (**Figures 4B**, **5B**, **6B**).

Rare Allele Loss

The average overall allele frequency across all alleles in the dataset before the eradication campaign was 0.164, meaning that each individual allele constituted, on average, 16.4% of the alleles observed across all genotypes at a locus. The pre-eradication frequency of just the alleles that dropped out after the eradication campaign was much lower at 0.037. This indicates that rare alleles were much more likely to be lost from the population, likely through genetic drift. Though it should be noted they may have been present in the population, but went undetected in our later sampling (i.e., if those alleles were not present in our sampled rats).

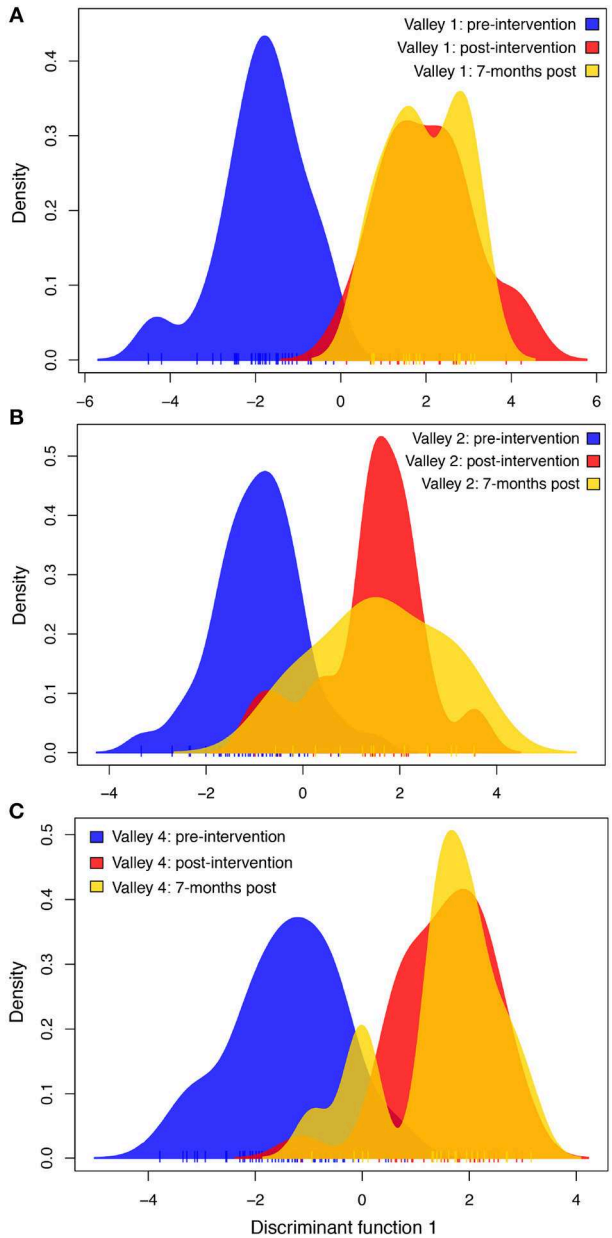

FIGURE 3 | Discriminant analysis of principal components (DAPC) for **(A)** Valley 1, **(B)** Valley 2, and **(C)** Valley 4 at three time points—immediately before the chemical intervention campaign, 1 month following the campaign, and 7 months following the campaign. Each plot displays the first discriminant function on the *x*-axis, and the density of genotypes on the *y*-axis. Increasing distance on the *x*-axis between peak densities represents increasing genetic differences between samples at those time points. In all three valleys, the mean genetic signature of rats shifted after the chemical intervention (i.e., separation between blue curve and the red and yellow post-intervention curves). This difference persisted over the 7 months after the intervention campaign, despite demographic changes in the populations.

DISCUSSION

Cities around the world have implemented intervention campaigns to reduce rat population sizes, usually in the form of lethal trapping or chemical eradication campaigns. But the genetic impacts of the control efforts on the remaining rat populations have not received much research attention. Here we find that an eradication campaign in Salvador, Brazil conducted in 2015 had rapid and severe genetic consequences for rats in three sections of the Pau da Lima favela. The effective population size declined between 85 and 91% after the eradication across the three sites (**Figure 2**), concurrent with a large shift in genetic structure (**Figure 3**) and the loss of dozens of alleles that had been at low frequency in the population. The levels of relatedness among the rats also increased significantly after eradication for two of the three locations. These data suggest that there are very likely to be long-term evolutionary implications to rat populations subject to control measures, even if their census numbers rebound quickly following the intervention campaigns.

Rapid Genetic Bottlenecks During Lethal Control

Effective population size (N_e) is a comprehensive measure of genetic variation and the loss of such variation results from drift. Importantly it provides a much better measure of the long-term genetic viability of a population than census population size, accounting for the genetic variation available to serve as the grist of future evolution to changing conditions (Dlugosch and Parker, 2008). If genetic variation in the population is severely decreased during a rat-control campaign, the effective population size would also decrease relative to pre-eradication numbers (Vucetich et al., 1997). The magnitude of this decline indicates how severely the gene pool of the rat population was impacted by the control campaign.

In Salvador, the N_e dropped 42–74% between the pre- and post-eradication campaign sampling periods using the sibship frequency method. All three valleys show immediate drops in N_e 1 month after the eradication, and these declines persisted or further decreased 7 months post-eradication (**Figure 2A**). The reduction in N_e was much sharper using the linkage-disequilibrium approach, falling 85–91% between the pre- and post-eradication periods (**Figure 2B**). However, in Valley 4 the decrease was not seen until 7 months post-campaign.

Despite the "boomerang effect" commonly seen when rat population sizes rebound after the control campaign has ended (Smith, 1963; de Masi et al., 2009), genetic variation lost during these bottleneck events takes much longer to return, through a combination of immigration and new mutations arising (Nei et al., 1975). If an urban rat population is repeatedly subjected to bottlenecks in population size—and the attendant genetic bottlenecks—during frequent control campaigns, the levels of genetic variation lost should accumulate during each successive bottleneck. However, the drastic reduction in N_e we observed (42–91%, depending on measure and valley) in Salvador with just a single lethal-control campaign suggests that genetic variation is lost exceedingly fast. This would leave very little genetic variation in the populations for future evolution, and increases the risk of deleterious fitness consequences from genetic drift and inbreeding depression (Briskie and Mackintosh, 2004; Spielman et al., 2004).

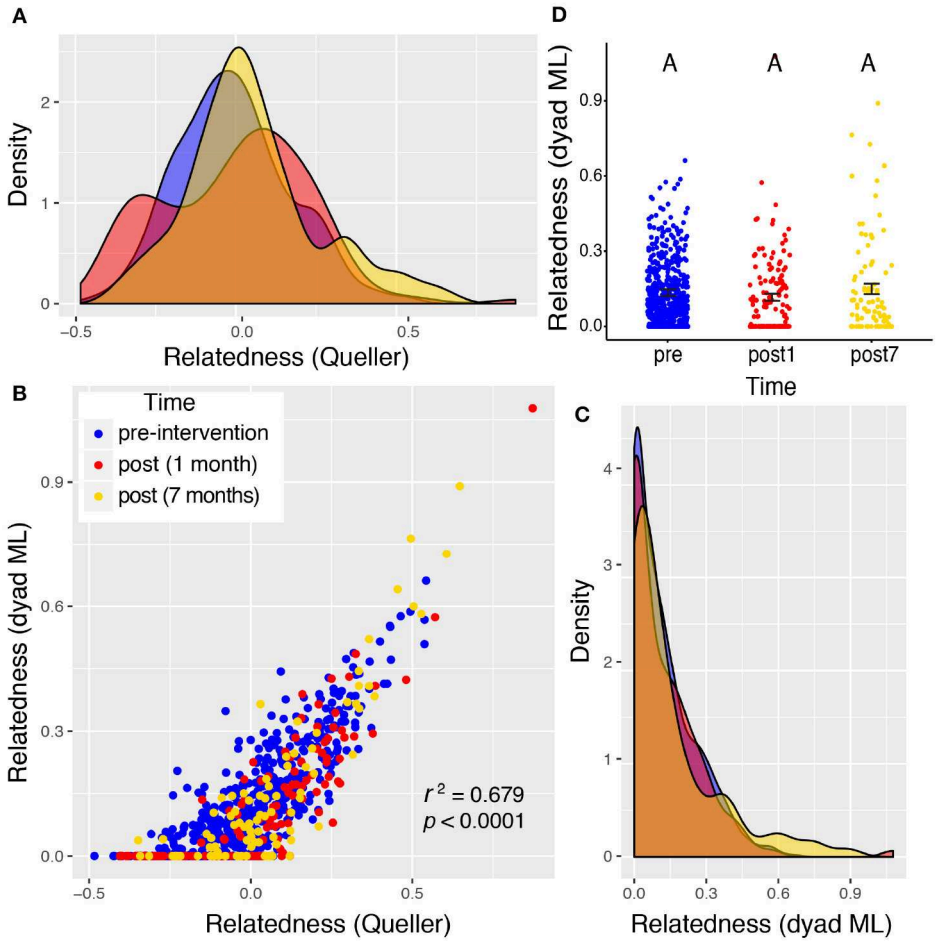

FIGURE 4 | Valley 1: two very different measures of relatedness suggest that there was no significant change in the distribution of relatedness between individual rats sampled pre- and post-eradication in Valley 1, represented by density distributions below **(A,C)**. The Queller-Goodnight relatedness coefficient can take negative values **(A)**, while the Milligan dyad maximum likelihood (dyad ML) metric is bounded by zero and one **(C)**. The two metrics are tightly correlated in this data set **(B)**. A Kruskal–Wallis test was used to determine if the distribution of relatedness values differed among the three time points sampled around the 2015 eradication campaign **(D)**. The upper-case letters above the points in panel **(D)** are all the same, indicating that there is no significant difference between the distributions of relatedness pre- and post-eradication. Black bars represent the mean ± 1SE.

Targeting the genetic variation of rat populations with the goal of increasing the risks associated with inbreeding, depression may be one avenue for urban pest control and integrated pest management (IPM) strategies (Hone, 1995; Kuriwada et al., 2010; Li et al., 2015). However, this is the same fundamental genetic process observed when a small number of founder individuals colonize a new area or island, with little evidence of deleterious effects on population growth from these founder effects, as seen with invasive rats and birds on islands (Abdelkrim et al., 2005; Low et al., 2018). Little research has been done on the relative fitness of urban rats, let alone how genetics factors into that fitness. It is possible that rat populations do suffer some fitness consequences after control campaigns reduce population sizes and allelic diversity. For example, both selection and drift can play a role in rare allele loss for important immune response loci in the major histocompatibility complex (MHC) (Sutton et al., 2011; Taylor et al., 2012). Alternatively, strong natural selection in cities may have quickly purged deleterious alleles as part of, or independent from, eradication campaigns.

Disruption of Population Genetic Structure

The eradication campaign in 2015 led to a large, abrupt shift in population genetic structure of the rats in all three valleys (**Figure 3**). This change in the genetic signature persisted or increased between the 1 and 7-months post-eradication sampling periods, as denoted along the discriminant function axis of the DAPC plot (**Figure 3**). This population genetic shift indicates that the genetic variation remaining after the eradication bottleneck is not a random subset of the gene pool present before the campaign. Population genetic structure around an eradication event has not received much research attention. However, Abdelkrim et al. (2007) used F-statistics and assignment probabilities (as measures of population structure) of individual rats remaining after an eradication of rats from

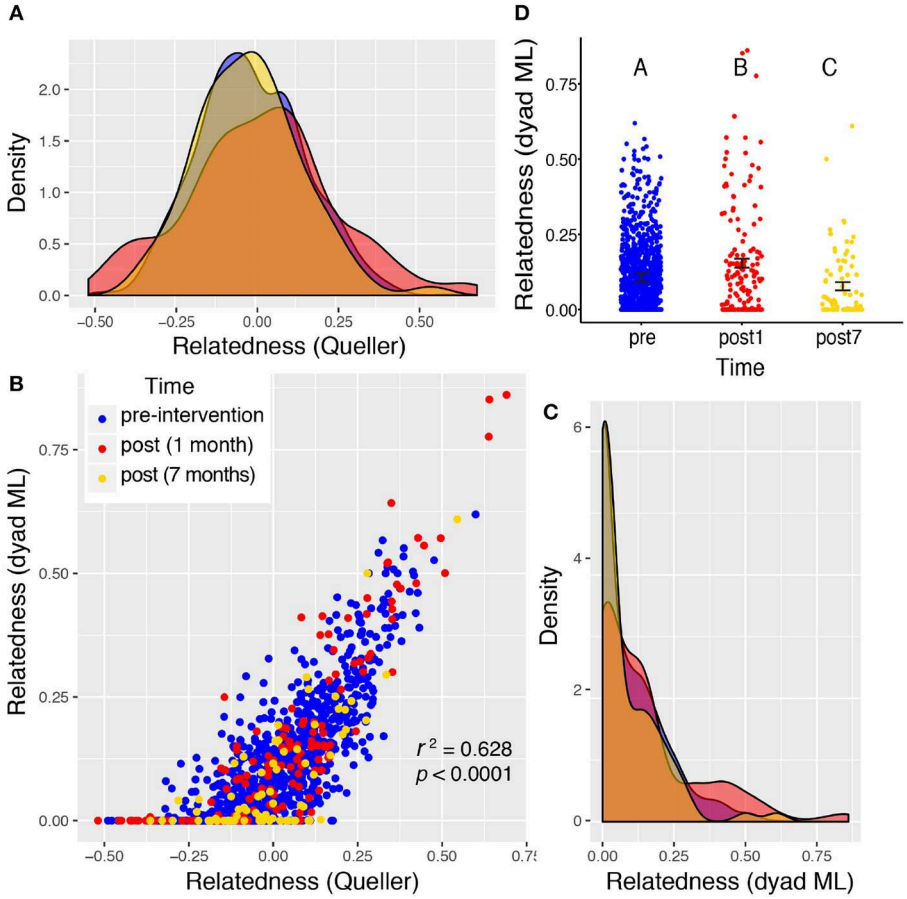

FIGURE 5 | Valley 2: two very different measures of relatedness suggest that there is a significant change in the distribution of relatedness between individual rats sampled pre- and post-eradication in Valley 2, represented by density distributions below **(A,C)**. The Queller-Goodnight relatedness coefficient can take negative values **(A)**, while the Milligan dyad maximum likelihood (dyad ML) metric is bounded by zero and one **(C)**. The two metrics are tightly correlated in this data set **(B)**. A Kruskal–Wallis test was used to determine if the distribution of relatedness values differed among the three time points sampled around the 2015 eradication campaign **(D)**. The upper-case letters above the points in panel **(D)** indicate that there is a significant difference between all three distributions of relatedness among the one pre- and two post-eradication periods. Black bars represent the mean ± 1SE.

two islands off the coast of Martinique, and found varying degrees of genetic structure and an increase in genetic diversity, suggesting a role for both internal rebound and recolonization on the two islands. Russell et al. (2010) compared the genetics of 51 samples from two species of rats on two islands near New Zealand, and found that the genetic structure before and after the control was consistent with those rats reinvading from the larger neighboring island, rather than internal rebound. Yet both internal rebound and new invasions from a mainland population played a role in increasing rat numbers on another small island after a 2012 control campaign (Pichlmueller and Russell, 2018). We extend this previous work on islands to an urban context, using DAPC and a large sample of 241 rats collected around an intense eradication campaign. We highlight the rapid shifts in population genetic structure possible with an intense population bottleneck. To explore the relative roles of internal rebound vs. outside dispersers in repopulating the area, we also conducted a supplemental DAPC analysis that included all rats from each valley at each time point. This analysis showed that valleys became more different genetically after the eradication campaign (**Figure S1**), indicating that rebounding rats are unlikely to be the result of large-scale immigration of dispersing rats from other, genetically dissimilar, areas.

Increasing Relatedness and Fitness Consequences

Norway rats exhibit a colonial social structure, where individuals in a colony are more closely related to each other than rats across the rest of the population (Costa et al., 2016). Given this social context, individuals may exhibit higher levels of relatedness after an eradication campaign if some colonies survive at higher rates and the residual breeding pairs are more related to each other than pre-eradication (i.e., a precursor to inbreeding). For example, if lethal baiting and trapping is deployed in a way that does not uniformly cover the focal area, this can lead to differential survival among colonies. Alternatively, if the eradication impacted all

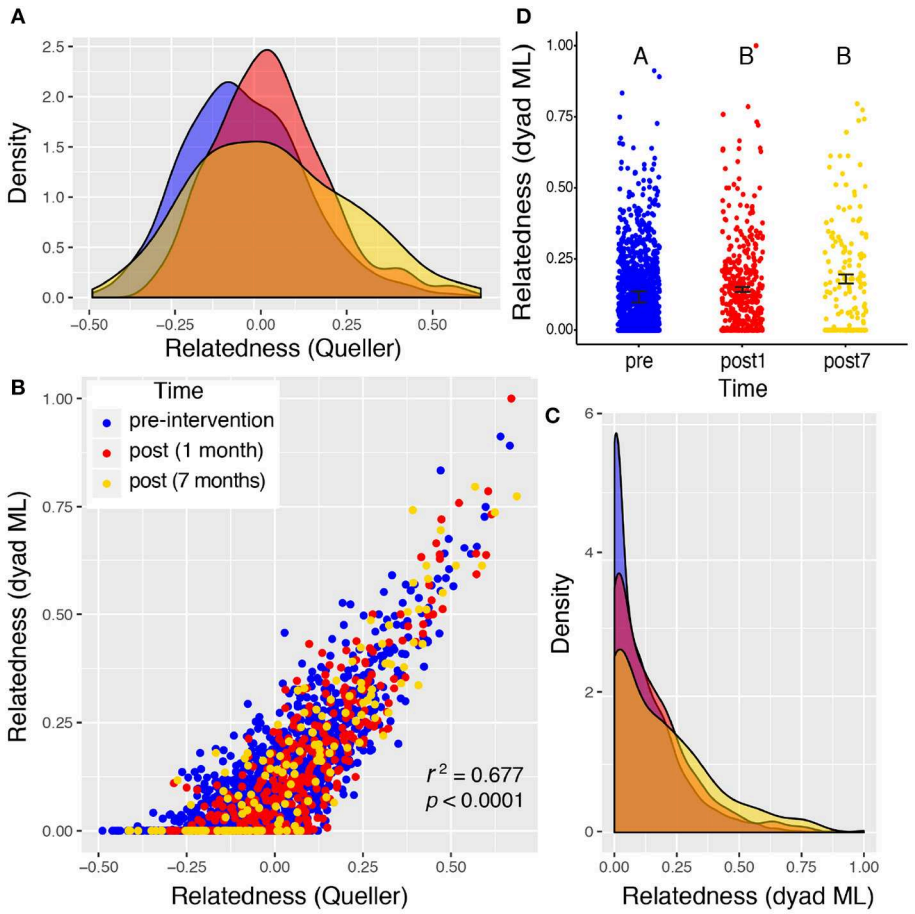

FIGURE 6 | Valley 4: two very different measures of relatedness suggest that there is a significant change in the distribution of relatedness between individual rats sampled pre- and post-eradication in Valley 4, represented by density distributions below **(A,C)**. The Queller-Goodnight relatedness coefficient can take negative values (a), while the Milligan dyad maximum likelihood (dyad ML) metric is bounded by zero and one **(C)**. The two metrics are tightly correlated in this data set **(B)**. A Kruskal–Wallis test was used to determine if the distribution of relatedness values differed among the three time points sampled around the 2015 eradication campaign **(D)**. The upper-case letters above the points in panel **(D)** indicate that there is a significant difference between the distributions of relatedness from the pre- and post-eradication periods, but the two post-eradication periods are not significantly different from each other. Black bars represent the mean ± 1SE.

areas and colonies equally, then relatedness would not be expected to increase initially, though a gene pool with less overall genetic variation could still impact the fitness of the remaining population. The rodenticide application in 2015 in Pau da Lima was designed to be uniformly distributed across Valleys 1 and 4, with incidental application in Valley 2 based on leptospirosis risk. In practice, logistics associated with access to households and permission of residents may have led to deviations in the intended uniform distribution of the rodenticides applied. Logistical constraints are a reality in any urban control effort.

Relatedness levels increase in all three valleys after the eradication campaign. The distribution of relatedness values (across all pairs of rats within a sampling period) increased significantly in the post-eradication rat population for valleys 2 and 4 (**Figures 5**, **6**). This same trend was seen in Valley 1, but not statistically significant (**Figure 4**). This pattern occurred using both the dyad maximum likelihood and the Queller & Goodnight metrics of relatedness, and those two metrics were tightly associated with each other (**Figures 4B**, **5B**, **6B**). The elevated relatedness among rats remaining after the control campaigns represents another risk for inbreeding in these post-eradication populations. Increasing relatedness can have fitness consequences, and has been found to reduced reproductive success, competitive ability and growth rates in invasive plant species (Burns, 2006; Elam et al., 2007). Yet there is evidence that some social animals prefer inbreeding despite documented deleterious impacts, such as elevated susceptibility to disease in American crows (Townsend et al., 2018). This raises the question of what tradeoffs exist between tight social relationships, inclusive fitness, and inbreeding depression. It is possible that urban rats benefit from sharing colonies with closely related individuals (e.g., territory defense, stable social structure), and may avoid some inbreeding tradeoffs by having relatively accelerated reproductive life cycles. One benefit may be less diverse parasite and pathogen communities harbored by isolated rats, and dispersal and culling can disrupt these relatively insular colonies (Angley et al., 2018; Lee et al., 2018; Minter et al., 2019).

Implications for Urban Rat Management

The current study is part of a multi-disciplinary effort to reduce the risk of leptospirosis for Pau da Lima residents, and a primary strategy is to reduce the population of Norway rats that serve as reservoirs of this bacterial pathogen (Ko et al., 1999; Costa et al., 2014; Richardson et al., 2017). Cities around the world are focused on reducing rat populations for reasons related to public health, quality of life, and protection of infrastructure (Bonnefoy et al., 2008; de Masi et al., 2009; Angley et al., 2018). The lethal control campaigns typically implemented are often successful at reducing the number of rats in the targeted areas yet are generally followed by a period of rapid population increase—the "boomerang effect" (Smith, 1963; de Masi et al., 2009). For that reason, a multifaceted IPM approach is needed to have longer-lasting impacts in reducing rat numbers. In addition to the lethal removal of rats, the amount of trash and other food sources need to be reduced. Habitat that can serve as harborage for rats also need to be managed, and infrastructure needs to be improved to prevent them from entering buildings.

The results of the current study also indicate that long-term genetic viability of urban rat populations may be another component relevant for any IPM strategy. If a single eradication campaign in Salvador can result in up to a 91% decline in effective population size, significant increases in relatedness, and the loss of dozens of rare alleles, then repeated campaigns may leave the remaining rat population genetically depauperate enough that severe inbreeding depression will eventually impart substantial fitness consequences. Over time, reduced fitness should erode a population's ability to reproduce at high rates and rebound after the bottleneck. These genetic impacts may be attenuated if immigration occurs from other populations with distinct genetic signatures, though previous research did not find evidence of this dispersal into Pau da Lima (Richardson et al., 2017).

In a larger evolutionary sense, repeated bottlenecks will leave less genetic variation available for the population to evolve and adapt in response to changing environments. This may be a particularly important handicap for urban-dwelling rodents, because cities impose strong and diverse natural selection pressures known to impact rodents (Donihue and Lambert, 2015; Harris and Munshi-South, 2017; Johnson and Munshi-South, 2017) and at the microgeographic spatial scales relevant for heterogeneous urban environments (Richardson et al., 2014). Genetic insights into rats provide another important dimension to our understanding of their use of and movement through the urban landscape, especially when compared across multiple cities (Richardson et al., 2016; Combs et al., 2018). For that reason, we recommend that genetic impacts on, and long-term viability of urban pest species, be considered as part of any comprehensive intervention campaign and IPM strategy.

ETHICS STATEMENT

The ethics committee for the use of animals from the Oswaldo Cruz Foundation, Salvador, Brazil approved the protocols used in this study (protocol number 003/2012), which adhered to the guidelines of the American Society of Mammalogists for the use of wild mammals in research and the guidelines of the American Veterinary Medical Association for the euthanasia of animals. These protocols were also approved by the Yale University Institutional Animal Care and Use Committee (IACUC), New Haven, Connecticut (protocol number 2012–11498).

AUTHOR CONTRIBUTIONS

FC, AK, JC, AC, and JR designed the study. AP, CM, IS, AZ, TC, and JR conducted trapping, genetic assays, and data collection. JR and GS analyzed the data. JR wrote the article with input from FC, AC, AK, JC, and GS.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00115/full#supplementary-material

REFERENCES

Abdelkrim, J., Pascal, M., Calmet, C., and Samadi, S. (2005). Importance of assessing population genetic structure before eradication of invasive species: examples from Insular Norway rat populations. *Conserv. Biol.* 19, 1509–1518. doi: 10.1111/j.1523-1739.2005.00206.x

Abdelkrim, J., Pascal, M., and Samadi, S. (2007). Establishing causes of eradication failure based on genetics: case study of ship rat eradication in Ste. Anne archipelago. *Conserv. Biol.* 21, 719–730. doi: 10.1111/j.1523-1739.2007.00696.x

Angley, L. P., Combs, M., Firth, C., Frye, M. J., Lipkin, I., Richardson, J. L., et al. (2018). Spatial variation in the parasite communities and genomic structure of urban rats in New York City. *Zoonoses Public Health* 65, e113–e123. doi: 10.1111/zph.12418

Bonnefoy, X., Kampen, H., and Sweeney, K. (2008). *Public Health Significance of Urban Pests*. Copenhagen: World Health Organization (WHO).

Briskie, J. V., and Mackintosh, M. (2004). Hatching failure increases with severity of population bottlenecks in birds. *Proc Natl Acad Sci U.S.A.* 101, 558–561. doi: 10.1073/pnas.0305103101

Burns, J. H. (2006). Relatedness and environment affect traits associated with invasive and noninvasive introduced Commelinaceae. *Ecol. Appl.* 16, 1367–1376. doi: 10.1890/1051-0761(2006)016[1367:RAEATA]2.0.CO;2

Calhoun, J. B. (1963). *The Ecology and Sociology of the Norway rat*. Bethesda, MD: U.S. Dept. of Health, Education, and Welfare, Public Health Service. doi: 10.5962/bhl.title.112283

Capizzi, D., Bertolino, S., and Mortelliti, A. (2014). Rating the rat: global patterns and research priorities in impacts and management of rodent pests. *Mamm. Rev.* 44, 148–162. doi: 10.1111/mam.12019

Clapperton, B. K. (2006). *A Review of the Current Knowledge of Rodent Behaviour in Relation to Control Devices*. Wellington: New Zealand Department of Conservation.

Combs, M., Byers, K. A., Ghersi, B. M., Blum, M. J., Caccone, A., Costa, F., et al. (2018). Urban rat races: spatial population genomics of brown rats (*Rattus norvegicus*) compared across multiple cities. *Proc. R. Soc. B Biol. Sci.* 285:20180245. doi: 10.1098/rspb.2018.0245

Costa, F., Ribeiro, G. S., Felzemburgh, R. D., Santos, N., Reis, R. B., Santos, A. C., et al. (2014). Influence of household rat infestation on leptospira transmission in the urban slum environment. *PLoS Negl. Trop. Dis.* 8:e3338. doi: 10.1371/journal.pntd.0003338

Costa, F., Richardson, J. L., Dion, K., Mariani, C., Pertile, A. C., Burak, M. K., et al. (2016). Multiple paternity in the Norway Rat, *Rattus norvegicus*, from Urban Slums in Salvador, Brazil. *J. Heredity* 107, 181–186. doi: 10.1093/jhered/esv098

Davis, H., Casta, A., and Schatz, G. (1977). *Urban Rat Surveys*. Washington, DC: US Department of Health, Education, and Welfare.

de Masi, E., Vilaça, P. J., and Razzolini, M. T. P. (2009). Evalua on on the effectiveness of actions for controlling infestation by rodents in Campo Limpo region, Sao Paulo Municipality, Brazil. *Int. J. Environ. Health Res.* 19, 291–304. doi: 10.1080/09603120802592723

Desvars-Larrive, A., Hammed, A., Hodroge, A., Berny, P., Benoît, E., LattardV., et al. (2018). Population genetics and genotyping as tools for planning rat management programmes. *J. Pest Sci.* 92, 691–705. doi: 10.1007/s10340-018-1043-4

Dlugosch, K. M., and Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* 17, 431–449. doi: 10.1111/j.1365-294X.2007.03538.x

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., and Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol. Ecol. Resour.* 14, 209–214. doi: 10.1111/1755-0998.12157

Donihue, C. M., and Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio* 44, 194–203. doi: 10.1007/s13280-014-0547-2

Easterbrook, J. D., Kaplan, J. B., Vanasco, N. B., Reeves, W. K., Purcell, R. H., Kosoy, M. Y., et al. (2007). A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, U. S. A. *Epidemiol. Infect.* 135, 1192–1199. doi: 10.1017/S0950268806007746

Elam, D. R., Ridley, C. E., Goodell, K., and Ellstrand, N. C. (2007). Population size and relatedness affect fitness of a self-incompatible invasive plant. *Proc. Natl. Acad. Sci. U.S.A.* 104, 549–552. doi: 10.1073/pnas.0607306104

Felzemburgh, R. D., Ribeiro, G. S., Costa, F., Reis, R. B., Hagan, J. E., Melendez, A. X., et al. (2014). Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the Leptospira agent. *PLoS Negl. Trop. Dis.* 8:e2927. doi: 10.1371/journal.pntd.0002927

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P., et al. (2014). Detection of zoonotic pathogens and characterization of novel viruses carried by commensal rattus norvegicus in New York city. *MBio* 5. doi: 10.1128/mBio.01933-14

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. *Genet. Res.* 66, 95–107. doi: 10.1017/S0016672300034455

Fraser, C. I., Banks, S. C., and Waters, J. M. (2015). Priority effects can lead to underestimation of dispersal and invasion potential. *Biol. Invasions* 17, 1–8. doi: 10.1007/s10530-014-0714-1

Gardner-Santana, L. C., Norris, D. E., Fornadel, C. M., Hinson, E. R., Klein, S. L., and Glass, G. E. (2009). Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*). *Mol. Ecol.* 18, 2766–2778. doi: 10.1111/j.1365-294X.2009.04232.x

Garza, J. C., and Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Mol. Ecol.* 10, 305–318. doi: 10.1046/j.1365-294x.2001.01190.x

Goudet, J., Kay, T., and Weir, B. S. (2018). How to estimate kinship. *Mol. Ecol.* 27, 4121–4135. doi: 10.1111/mec.14833

Hagan, J. E., Moraga, P., Costa, F., Capian, N., Ribeiro, G. S., Wunder, E. A., et al. (2016). Spatiotemporal determinants of Urban leptospirosis transmission: four-year prospective cohort study of slum residents in Brazil. *PLoS Negl. Trop. Dis.* 10:e0004275. doi: 10.1371/journal.pntd.0004275

Harris, S. E., and Munshi-South, J. (2017). Signatures of positive selection and local adaptation to urbanization in white-footed mice (*Peromyscus leucopus*). *Mol. Ecol.* 26, 6336–6350. doi: 10.1111/mec.14369

Hill, W. G. (1981). Estimation of effective population size from data on linkage disequilibrium. *Genet. Res.* 38, 209–216. doi: 10.1017/S0016672300020553

Himsworth, C. G., Bidulka, J., Parsons, K. L., Feng, A. Y., Tang, P., Jardine, C. M., et al. (2013a). Ecology of Leptospira interrogans in Norway rats (*Rattus norvegicus*) in an inner-City Neighborhood of Vancouver, Canada. *PLoS Negl. Trop. Dis.* 7:2270. doi: 10.1371/journal.pntd.0002270

Himsworth, C. G., Parsons, K. L., Jardine, C., and Patrick, D. M. (2013b). Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne Zoonotic Dis.* 13, 349–359. doi: 10.1089/vbz.2012.1195

Hone, J. (1995). Spatial and temporal aspects of vertebrate pest damage with emphasis on feral pigs. *J. Appl. Ecol.* 32:311.

Howald, G., Donlan, C. J., Galván, J. P., Russell, J. C., Parkes, J., Samaniego, A., et al. (2007). Invasive rodent eradication on Islands. *Conserv. Biol.* 21, 1258–1268. doi: 10.1111/j.1523-1739.2007.00755.x

Husemann, M., Zachos, F. E., Paxton, R. J., and Habel, J. C. (2016). Effective population size in ecology and evolution. *Heredity* 117, 191–192. doi: 10.1038/hdy.2016.75

Johnson, M. T. J., and Munshi-South, J. (2017). Evolution of life in urban environments. *Science* 358:eaam8327. doi: 10.1126/science.aam8327

Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* 11:94. doi: 10.1186/1471-2156-11-94

Jombart, T., Kamvar, Z. N., Lustrik, R., et al. (2016). *Adegenet: an R package for the Exploratory Analysis of Genetic and Genomic Data*. R package version 2.0.1.

Jones, O. R., and Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol. Ecol. Resour.* 10, 551–555. doi: 10.1111/j.1755-0998.2009.02787.x

Kajdacsi, B., Costa, F., Hyseni, C., Porter, F., Brown, J., Rodrigues, G., et al. (2013). Urban population genetics of slum-dwelling rats (*Rattus norvegicus*) in Salvador, Brazil. *Mol. Ecol.* 22, 5056–5070. doi: 10.1111/mec.12455

Kalinowski, S. T., and Waples, R. S. (2002). Relationship of effective to census size in fluctuating populations. *Conserv. Biol.* 16, 129–136. doi: 10.1046/j.1523-1739.2002.00134.x

Klaassen, C. H., Gibbons, J. G., Fedorova, N. D., Meis, J. F., and Rokas, A. (2012). Evidence for genetic differentiation and variable recombination rates among Dutch populations of the opportunistic human pathogen *Aspergillus fumigatus*. *Mol. Ecol.* 21, 57–70. doi: 10.1111/j.1365-294X.2011.05364.x

Ko, A. I., Reis, M. G., Dourado, C. M. R., Johnson, W. D., and Riley, L. W. (1999). Urban epidemic of severe leptospirosis in Brazil. *Lancet* 354, 820–825. doi: 10.1016/S0140-6736(99)80012-9

Kuriwada, T., Kumano, N., Shiromoto, K., and Haraguchi, D. (2010). Effect of mass rearing on life history traits and inbreeding depression in the sweetpotato Weevil (Coleoptera: Brentidae). *J. Econ. Entomol.* 103, 1144–1148. doi: 10.1603/EC09361

Lee, M. J., Byers, K. A., Donovan, C. M., Bidulka, J. J., Stephen, C., Patrick, D. M., et al. (2018). Effects of culling on leptospira interrogans carriage by rats. *Emerg. Infect. Dis.* 24, 356–360. doi: 10.3201/eid2402.171371

Li, S. P., Cadotte, M. W., Meiners, S. J., Hua, Z. S., Shu, H. Y., Li, J. T., et al. (2015). The effects of phylogenetic relatedness on invasion success and impact: deconstructing Darwin's naturalisation conundrum. *Ecol. Lett.* 18, 1285–1292.

Low, G. W., Chattopadhyay, B., Garg, K. M., Irestedt, M., Ericson, P., Yap, G., et al. (2018). Urban landscape genomics identifies fine-scale gene flow patterns in an avian invasive. *Heredity* 120, 138–153. doi: 10.1038/s41437-017-0026-1

Luikart, G., Allendorf, F. W., Cornuet, J. M., and Sherwin, W. B. (1998). Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Heredity* 89, 238–247. doi: 10.1093/jhered/89.3.238

Maruyama, T., and Fuerst, P. A. (1985). Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* 111, 675–689.

Milligan, B. G. (2003). Maximum-likelihood estimation of relatedness. *Genetics* 163, 1153–1167.

Minter, A., Himsworth, C. G., Byers, K. A., Childs J. E., Ko, A. I., and Costa, A. et al. (2019). Tails of two cities: age and wounding are associated with carriage of leptospira interrogans by Norway Rats (*Rattus norvegicus*) in ecologically distinct Urban environments. *Front. Ecol. Evol.* 7:14. doi: 10.3389/fevo.2019.00014

Murphy, R. G., and Oldbury, D. J. (2002). "Rat control by local authorities within the UK," in *Proceedings of the 4th International Conference on Urban Pests (ICUP),* eds S. C. Jones, J. Zhai, and W. H. Robinson (Charleston), 351–355.

Nei, M., Maruyama, T., and Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* 29, 1–10. doi: 10.1111/j.1558-5646.1975.tb00807.x

Panti-May, J. A., Carvalho-Pereira, T. S. A., Serrano, S., Pedra, G. G., Taylor, J., Pertile, A. C., et al. (2016). A two-year ecological study of Norway rats (*Rattus norvegicus*) in a Brazilian Urban Slum (G Chapouthier, Ed,). *PLoS ONE* 11:e0152511. doi: 10.1371/journal.pone.0152511

Paradis, E. (2010). pegas: an R package for population genetics with an integrated?modular approach. *Bioinformatics* 26, 419–420. doi: 10.1093/bioinformatics/btp696

Park, S. (2001). *The Excel Microsatellite Toolkit (v3.1)*. Animal Genomics Laboratory, UCD.

Parsons, M. H., Banks, P. B., Deutsch, M. A., Corrigan, R. F., and Munshi-South, J. (2017). Trends in urban rat ecology: a framework to define the prevailing knowledge gaps and incentives for academia, pest management professionals (PMPs) and public health agencies to participate. *J. Urban Ecol.* 3:jux005. doi: 10.1093/jue/jux005

Peery, M. Z., Kirby, R., Reid, B. N., Stoelting, R., Doucet-Bëer, E., Robinson, S., et al. (2012). Reliability of genetic bottleneck tests for detecting recent population declines. *Mol. Ecol.* 21, 3403–3418. doi: 10.1111/j.1365-294X.2012.05635.x

Pichlmueller, F., and Russell, J. C. (2018). Survivors or reinvaders? Intraspecific priority effect masks reinvasion potential. *Biol. Conserv.* 227, 213–218. doi: 10.1016/j.biocon.2018.09.020

Pimentel, D., Zuniga, R., and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52, 273–288. doi: 10.1016/j.ecolecon.2004.10.002

Piry, S., Luikart, G., and Cornuet, J.-M. (1999). Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. *J. Heredity* 90, 502–503. doi: 10.1093/jhered/90.4.502

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.

Queller, D. C., and Goodnight, K. F. (1989). Estimating relatedness using genetic-markers. *Evolution* 43, 258–275. doi: 10.1111/j.1558-5646.1989.tb04226.x

Reed, D. H. (2005). Relationship between population size and fitness. *Conserv. Biol.* 19, 563–568. doi: 10.1111/j.1523-1739.2005.00444.x

Reis, R. B., Ribeiro, G. S., Felzemburgh, R. D., Santana, F. S., Mohr, S., Melendez, A. X., et al. (2008). Impact of environment and social gradient on Leptospira infection in urban slums. *PLoS Negl. Trop. Dis.* 2:e228. doi: 10.1371/journal.pntd.0000228

Richardson, J. L., Brady, S. P., Wang, I. J., and Spear, S. F. (2016). Navigating the pitfalls and promise of landscape genetics. *Mol. Ecol.* 25, 849–863. doi: 10.1111/mec.13527

Richardson, J. L., Burak, M. K., Hernandez, C., Shirvell, J. M., Mariani, C., Carvalho-Pereira, T. S., et al. (2017). Using fine scale spatial genetics of Norway rats to improve control efforts and reduce leptospirosis risk in urban slum environments. Evol. Appl. 10, 323–337. doi: 10.1111/eva.12449

Richardson, J. L., Urban, M. C., Bolnick, D. I., and Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends Ecol. Evol.* 29, 165–176. doi: 10.1016/j.tree.2014.01.002

Russell, J. C., Miller, S. D., Harper, G. A., MacInnes, H. E., Wylie, M. J., and Fewster, R. M. (2010). Survivors or reinvaders? Using genetic assignment to identify invasive pests following eradication. *Biol. Invasions* 12, 1747–1757. doi: 10.1007/s10530-009-9586-1

Savidge, J. A., Hopken, M. W., Witmer, G. W., Jojola, S. M., Pierce, J. J., and Burke, P. W. (2012). Genetic evaluation of an attempted Rattus eradication on Congo Cay, U.S. Virgin Islands, identifies importance of eradication units. *Biol. Invasions* 14, 2343–2354. doi: 10.1007/s10530-012-0233-x

Smith, L. (1963). An experimental rat eradication program in an urban area. *Public Health Rep.* 78, 807–811. doi: 10.2307/4591943

Spielman, D., Brook, B. W., Briscoe, D. A., and Frankham, R. (2004). Does inbreeding and loss of genetic diversity decrease disease resistance? *Conserv. Genet.* 5, 439–448. doi: 10.1023/B:COGE.0000041030.76598.cd

Stenseth, N. C., Leirs, H., Skonhoft, A., Davis, S. A., Pech, R. P., Andreassen, H. P., et al. (2003). Mice, rats, and people: the bio-economics of agricultural rodent pests. *Front. Ecol. Environ.* 1, 367–375. doi: 10.1890/1540-9295(2003)001[0367:MRAPTB]2.0.CO;2

Stokes, V. L., Banks, P. B., Pech, R. P., and Spratt, D. M. (2009). Competition in an invaded rodent community reveals black rats as a threat to native bush rats in littoral rainforest of south-eastern Australia. *J. Appl. Ecol.* 46, 1239–1247. doi: 10.1111/j.1365-2664.2009.01735.x

Sutton, J. T., Nakagawa, S., Robertson, B. C., and Jamieson, I. G. (2011). Disentangling the roles of natural selection and genetic drift in shaping variation at MHC immunity genes. *Mol. Ecol.* 20, 4408–4420. doi: 10.1111/j.1365-294X.2011.05292.x

Taylor, S. S., Jenkins, D. A., and Arcese, P. (2012). Loss of Mhc and neutral variation in peary caribou: genetic drift is not mitigated by balancing selection or exacerbated by mhc allele distributions (A Stow, Ed.). *PLoS ONE* 7:e36748. doi: 10.1371/journal.pone.0036748

Townsend, A. K., Taff, C. C., Jones, M. L., Getman, K. H., Wheeler, S. S., Hinton, M. G., et al. (2018). Apparent inbreeding preference despite inbreeding depression in the American crow. Mol. Ecol.

Vadell, M. V., Cavia, R., and Suárez, O. V. (2010). Abundance, age structure and reproductive patterns of *Rattus norvegicus* and *Mus musculus* in two areas of the city of Buenos Aires. *Int. J. Pest Manag.* 56, 327–336. doi: 10.1080/09670874.2010.499479

Vucetich, J. A., Waite, T. A., and Nunney, L. (1997). Fluctuating population size and the ratio of effective to census population size. *Evolution* 51, 2017–2021. doi: 10.1111/j.1558-5646.1997.tb05123.x

Wang, J. (2004). Sibship reconstruction from genetic data with typing errors. *Genetics* 166, 1963–1979. doi: 10.1534/genetics.166.4.1963

Wang, J. (2009). A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Mol. Ecol.* 18, 2148–2164. doi: 10.1111/j.1365-294X.2009.04175.x

Wang, J. (2016). A comparison of single-sample estimators of effective population sizes from genetic marker data. *Mol. Ecol.* 25, 4692–4711. doi: 10.1111/mec.13725

Waples, R. S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci*. *Conserv. Genet.* 7, 167–184. doi: 10.1007/s10592-005-9100-y

Waples, R. S., and Do, C. (2008). LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Resour.* 8, 753–756. doi: 10.1111/j.1755-0998.2007.02061.x

Waples, R. S., and Do, C. (2010). Linkage disequilibrium estimates of contemporary N e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evol. Appl.* 3, 244–262. doi: 10.1111/j.1752-4571.2009.00104.x

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frontiers in Ecology and Evolution

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Optimal Control of Rat-Borne Leptospirosis in an Urban Environment

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Humans acquire leptospirosis through direct contact with animal reservoirs, or more commonly, contact with the environment contaminated with leptospires shed in animal urine. Reservoir populations can be difficult to control through rodenticide application, and resource reduction via habitat management is costly and logistically complicated to implement. When resources are limited, simulation of different combinations of control methods can inform their application in the field. Here we present a framework to find time-dependent control measures for rodent-borne leptospirosis using optimal control mathematical model theory. An age-structured model for leptospire infection in a Norway rat (*Rattus norvegicus*) population was developed, informed by empirical analyses of data from the city of Salvador, Brazil. We extended this model to include two temporary control measures, rodenticide, and resource reduction, and two permanent control measures, reducing rat carrying capacity and leptospire lifespan in the environment. Optimal control theory seeks the optimum time-dependent controls while taking into account both the cost of the control measures and the "cost" of infection. Multiple control scenarios and the predicted effect of the optimal controls on the population and infection dynamics are presented to illustrate the applications of combinations of temporary and permanent controls. Permanent controls lead to a reduction in prevalence of leptospiral carriage in the rodent population. However, temporary controls can also achieve a reduction in the number of infected rats low enough to reduce risk to humans. Although we focus our modeling on a well-studied species, the Norway rat, our approach can be applied to other disease systems with animal and environmental reservoirs to inform decisions to reduce the risk of human infection.

Keywords: leptospirosis, Norway rat, mathematical model, intervention, urban system, control, prevention

INTRODUCTION

Leptospirosis is one of the most widespread zoonoses, with over a million cases worldwide (Costa et al., 2015a). Generally, animals are ultimately responsible for the maintenance of zoonotic pathogens causing human disease, and control of the animal population is often the main target in reducing human infection. Removal of zoonotic reservoirs to reduce or prevent human risk of infection has previously been achieved for Hantavirus (Zhang et al., 2010) and visceral leishmaniasis (Ashford et al., 1998). However, while humans may acquire leptospire infection through direct contact with the animal reservoir, it more commonly occurs through contact with the environment (water and soil) contaminated with leptospires shed in animal urine. In the urban slums of Salvador, Brazil, the incidence of severe cases of human leptospirosis is high, 58.7 per 100,000 residents, with annual peaks in the rainy season (Reis et al., 2008). To prevent human infection, the cycle of infection must be broken. In terms of control, one crucial choice, therefore, is whether to target the animal or the environmental reservoir.

Typical of tropical urban slum environments world-wide, Norway rats, *Rattus norvegicus*, are the natural reservoir of leptospirosis in Salvador (Costa et al., 2014b). The high prevalence of infection in the rat population (Costa et al., 2014a), the high concentration of leptospires shed by the rats (Costa et al., 2015b), and an apparent lifetime of shedding following infection (Ellis, 2015) combine to make Norway rats a particularly effective reservoir. Indeed, in Salvador, an environmental reservoir of soil and water contaminated with leptospires appears to be maintained solely by the Norway rat population (Costa et al., 2015b; Minter et al., 2017). Human leptospirosis in Salvador appears to be solely caused by *L. interrogans* serogroup Icterohaemorrhagiae. Such pathogenic leptospires are not known to reproduce in the environment, and survival at least of the vast majority of the population is short (Evangelista and Coburn, 2011; Casanovas-Massanaa et al., 2018). Therefore, reduction in the rodent population will, in turn, reduce the environmental load of leptospires.

Indirect evidence suggests that humans acquire infection predominantly through contact with open sewers and annual flooding washing contaminated soil into areas of human use (Reis et al., 2008; Hagan et al., 2016). Nonetheless, the question remains as to whether control should target the ultimate source of infection (the rats) or the immediate threat (typically, the contaminated environment).

Environmental controls, such as closure of open sewers and improved drainage, would directly reduce risk of infection for humans, by limiting their exposure to a contaminated environment, but require large scale concerted effort, making them more difficult, and expensive to implement than animal control (Costa et al., 2017). However, Norway rat populations are themselves difficult to control as they are neophobic (Clapperton, 2006), reducing uptake of rodenticides, and rats can become resistant to rodenticides (Clapperton, 2006). Populations recover quickly after a population decrease (Shilova and Tchabovsky, 2009), as a result either of *in situ* survival with subsequent reproduction or immigration (Hein and Jacob, 2015). At best, therefore, rodenticide exerts only short-term population reduction.

Reducing the suitability of habitat by restricting access to food and refuges can itself reduce the carrying capacity of the rodent population (Lambert et al., 2008; Adrichem et al., 2013; Buckle, 2013). For example, in cities like Salvador, reducing access to food could be achieved by improving removal of garbage and reducing access of rats to houses, and available refuges could be reduced by clearing larger pieces of garbage, construction materials, and dense vegetation. Further, the reduction of suitable rodent habitat by, for example, closing open sewers, would at the same time increase the mortality of leptospires by decreasing moisture levels in the soil and exposing them to UV radiation (Lambert et al., 2008; Casanovas-Massanaa et al., 2018). Additional environmental controls exist, such as paving areas to improve drainage, which we anticipate would increase leptospire mortality.

Mathematical models can be used to test the effectiveness of control measures in an infected population (Hethcote, 2000). Optimal control theory seeks to identify optimum time-dependent controls, while considering both the cost of the control measures and the cost of having no control (in this case the cost of rats, or of a given level of environmental contamination). Time-dependent effects are important as, for example, control measures applied intermittently (such as rodenticides) may be wasteful if applied at the wrong time, and reactive measures generally should be sensitive to changes in rat populations and the environment (Traweger et al., 2006). Given restrictions on resources and time, it is of interest to find the optimal level and disposition of control efforts to inform intervention policies.

Here, an age-structured model for leptospire infection in the Norway rat population of Salvador is presented, informed by empirical analysis (Costa et al., 2015b; De Oliveira et al., 2016; Panti-May et al., 2016; Minter et al., 2017). This model is then extended to include time dependent rodent control measures integrating rodenticide applications and resource reduction. We present the predicted effects of the control measures on the total population size of rats, the population size of infected individuals, and the size of the environmental load of leptospires. In addition, we explore the effects of environmental controls that permanently change the carrying capacity of the rodent population, the environmental loads and the mortality of leptospires.

Estimates of the costs of control measures, and especially of not applying controls, are all approximate. Hence, the outputs of our analysis are not designed as the basis for management recommendations. Rather, they provide illustrations of how the key features a rodent-environment-zoonosis system, exemplified by urban leptospirosis carried by rats, may drive the generation of optimal control strategies. Thus, for example, we explore how the timing of temporary measures (such as direct rodent control) may interact with more permanent interventions (for example, modifying the environment), and the dependence of this on their relative costs. Such general patterns may themselves then identify where improved estimates of costs would be most valuable in

the direct application of approaches such as this in developing management plans.

MATERIALS AND METHODS

An Age Structured Model for Leptospire Infection in *Rattus norvegicus*

The model is based on that presented by Minter et al. (2018) with an age structure following Holt et al. (2006). The model is assumed to represent a population of rodents within the urban slums of Salvador, Brazil as described in Minter et al. (2018). The urban slums are comprised of valleys in which the trapped population of rodents surpasses 100. It comprises a system of seven differential equations representing the number of juveniles (J), sub-adults (W), and adults (A) as follows:

$$\frac{dJ_X}{dt} = b(1-u(t))(A_X + (1-\upsilon_1)A_Y)\,exp(-\alpha(W+A)) - \varphi_J J_X - m_J J_X \quad (1)$$

$$\frac{dJ_Y}{dt} = b(1-u(t))A_Y\upsilon_1 exp(-\alpha(W+A)) - \varphi_J J_Y - m_J J_Y \quad (2)$$

$$\frac{dW_X}{dt} = \varphi_J J_X - \upsilon_2 W_X(W_Y+A_Y)/(W+A) - \upsilon_3 W_X L - \varphi_W W_X - m_W W_X - p\tau(t)\,W_X \quad (3)$$

$$\frac{dW_Y}{dt} = \varphi_J J_Y + \upsilon_2 W_X(W_Y+A_Y)/(W+A) + \upsilon_3 W_X L - \varphi_W W_Y - m_W W_Y - p\tau(t)\,W_Y \quad (4)$$

$$\frac{dA_X}{dt} = \varphi_W W_X - \upsilon_2 A_X(W_Y+A_Y)/(W+A) - \upsilon_3 A_X L - m_A A_X - p\tau(t)\,A_X \quad (5)$$

$$\frac{dA_Y}{dt} = \varphi_W W_Y + \upsilon_2 A_X(W_Y+A_Y)/(W+A) + \upsilon_3 A_X L - m_A A_Y - p\tau(t)\,A_Y \quad (6)$$

$$\frac{dL}{dt} = l_W W_Y + l_A A_Y - \mu L \quad (7)$$

with subscripts X and Y indicating susceptible and infected individuals, respectively (see also **Figure 1**).

Rats are born into the juvenile class at a constant rate b throughout the year. All offspring of susceptible adults (A_X) are born susceptible, but infected adults (A_Y) "give birth"

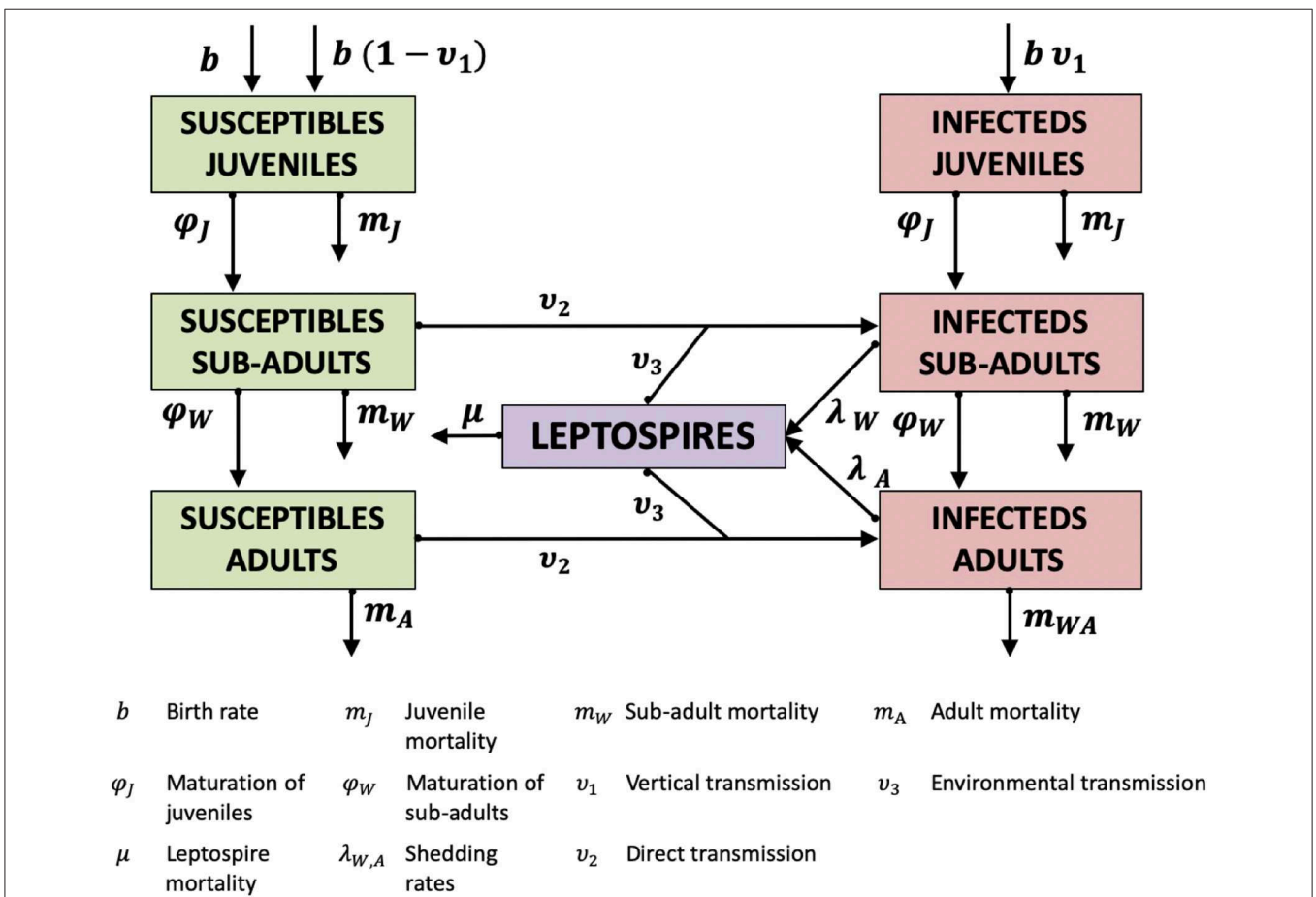

FIGURE 1 | Flow diagram of the age structured model with self-regulation incorporated.

TABLE 1 | Parameter definitions and values for the age-structured model.

Parameter	Definition	Units	Value	Source/Comments
b	Per capita birth rate	Day^{-1}	0.285	Panti-May et al., 2016
m_J	Juvenile rat mortality rate	Day^{-1}	0.125	High juvenile mortality (Calhoun, 1962)
m_W	Sub-adult rat mortality rate	Day^{-1}	0.013	Average lifespan is 125 days, most animals survive to mature into adults (Glass et al., 1989)
m_A	Adult rat mortality rate	Day^{-1}	0.015	Average lifespan 66 days (Glass et al., 1989)
φ_J	Maturation rate of juveniles	Day^{-1}	0.03	Average time spent in the nest 27 days (field data and Galef, 1981)
φ_W	Maturation rate of sub-adults	Day^{-1}	0.029	Average time to sexual maturity outside the nest is 50 days (field data and Calhoun, 1962; Villafañe et al., 2013)
υ_1	Proportion of pups infected from suckling and born infected	Day^{-1}	0.2	Probability of infection at 27 days is 0.2 (Minter et al., 2017)
υ_2	Transmission rate via direct transmission	Day^{-1}	0.0001	Assumed to occur at a low rate (Minter et al., 2017)
υ_3	Transmission via the environment	Day^{-1}	0.000003	Chosen to achieve comparable prevalence predictions. Assumed rate
$l_{W,A}$	Leptospires shed per day per infected sub-adult, adult.	Log10 scale, Day^{-1}	Log10 (1.6×10^7), Log10 (8.1×10^8)	Estimated from the median genome-equivalents in urine (Costa et al., 2015b)
μ	Mortality rate of leptospires in the environment	Day^{-1}	0.05	Lifespan of 20 days, (Casanovas-Massanaa et al., 2018)
α	Shape parameter for self-regulation	–	0.013	Achieves population size similar to estimates from field data (Pedra et al., unpublished)
p	Probability of rodents contacting rodenticide	Day^{-1}	0.2	Estimated from rodenticide application in the field (unpublished observations)

to a proportion (υ_1) of infected offspring. The offspring can be infected *in utero*, through perinatal infection or from environmental contamination in the nest. We cannot distinguish these routes as juvenile animals are confined to the nest. There is self-regulation of the birth rate, at intensity α, where all sub-adults and adults ($W + A = (W_X + W_Y) + (A_X + A_Y)$) are competing for resources, reducing the birth rate in the system, which in turn introduces an effective "carrying capacity" to the population. The parameter u relates to control and is described below.

Juveniles (J) are those individuals not yet able to exist independently outside the nest, suffering in-nest mortality at rate m_J. Juveniles mature into sub-adults at a rate φ_J. Sub-adults can become infected via direct contact with infected sub-adults or adults at rate (υ_2) or via contact with the environment (rate υ_3). Sub-adults suffer mortality at rate m_W. Sub-adults then mature into adults at a rate φ_W, where they are at risk of further direct and environmental transmission (at the same rates as the sub-adults, υ_2, υ_3). Adults suffer mortality at rate m_A.

Infected sub-adults and adults both shed into the population of free-living leptospires [L, Equation (7)], but at different rates (l_W, l_A) specified on a log10 scale. Infected juveniles may shed but if they do it will be in the nest, not into the environment as we have defined it here. Free-living leptospires suffer mortality at rate μ.

Parameter values were informed directly from field data or estimated based on field data. **Table 1** provides details.

Control Measures

We investigate the effect of, in total, four control measures on the rodent infection dynamics and environmental load of leptospires. These are two-time dependent controls—rodenticide and resource reduction—and two permanent environmental controls: carrying capacity control and leptospire mortality control. It is worth noting that both rodenticide and resource reduction will target all rats, not just those that are infected. Resource reduction can be implemented after a rodenticide program, the aim being to prevent the population from recovering. The time-dependent controls are incorporated into the structure of the mathematical model. The two environmental controls are assumed to have a permanent effect and so are specified as permanent changes in the model parameter values.

Rodenticide is incorporated by assuming that a proportional number of susceptible and infected, sub-adults and adults are removed, according to the total target proportion at time t, $\tau(t)$, and the probability that a rat contacts the rodenticide, p, which is constant (Equations 1–7). We assume that if a rodent contacts rodenticide, then death is certain, since, for example, Mlynarèíková et al. (1999) found a mortality rate of 100% after 8 days when Norway rats consumed bromadiolone rodenticide. Rodenticide is placed outside houses and so animals that are confined to the nest (juveniles) will not be affected.

Resource reduction is assumed to affect the rodent population by reducing the effective birth rate either by decreasing suitable habitat for nesting or reducing the resources available for reproduction. The control is formulated in the model by

assuming that the birth rate (b) is reduced by a proportion, $1 - u(t)$, at time t.

For the two permanent, environmental controls, we include, first, a carrying capacity control that permanently reduces habitat suitability for rats and hence affects the availability of the resources. This is specified as a reduction (ω_1) in the self-regulation parameter such that if we write $\alpha = 1/a$ then $\alpha^{new} = 1/(a(1-\omega_1))$. The second environmental control permanently reduces the suitability of the environment for leptospires, and so reduces their lifespan. This control is specified as $\frac{1}{\mu^{new}} = \frac{1}{\mu}(1 - \omega_2)$.

Given that there is no recovery, infection becomes endemic in the rodent population and the population sizes remain constant. When the model is run using the parameter values as specified in **Table 1** with no control, and endemic low prevalence (17%) is observed in the juveniles as the only transmission route to them is vertical. Prevalence reaches 56% in the sub-adult population and 87% in the adult population. In the free roaming population (sub-adults and adults combined) the prevalence is 71%. The proportion of animals in each category was 0.32 juveniles, 0.23 sub-adults, and 0.45 adults of a total population of ∼100. These endemic state values were used as the starting conditions for all control model simulations.

Optimal Control

Rats can be infected at any point in their lifetime, and so we wish to investigate the effect on the risk of human infection of reducing rat abundance overall. Hence, the time dependent control measures in the age-structured model (Equations 1–7) target all rats (susceptible and infected). Optimal control theory can be used to find the optimum amount of a time dependent control given restrictions on cost and on the length of the intervention programme (Sharomi and Malik, 2015). In the following sections, details of the optimal control problem are presented (see **Supplementary Information** for a brief introduction to optimal control and Sharomi and Malik, 2015 for examples in epidemiology).

The optimal control scheme is found by minimizing the so-called objective function. We aimed to reduce the total number of rats $H(t) = J_X(t) + J_Y(t) + W_X(t) + W_Y(t) + A_X(t) + A_Y(t)$ and the number of leptospires (on the log10 scale) while simultaneously minimizing the control efforts used. Here, these are the proportion of the rodent population targeted with rodenticide (τ) and the proportional reduction in the birth rate (u). Hence the objective function includes the total number of rats and two controls,

$$\int_{t_0}^{t_f} c_1 H(t) + c_2 L(t) + \frac{c_3}{2}\tau(t)^2 + \frac{c_4}{2}u(t)^2 \, dt \qquad (8)$$

where c_1, c_2, c_3, and c_4 are the weights which transform the component parts of the integral to the same monetary scale (**Table 2**). The weights are often specified in monetary terms, but given that the costs of these controls are unknown in absolute terms, we refer to the costs, for this initial exploration of this method, as "weights."

TABLE 2 | Value of fixed weights of rats, leptospires and the control measures.

Parameter	Definition	Value
c_1	Weight per rat	1
c_2	Weight per log10 leptospires	0.1
c_3	Weight per target proportion squared ($\tau(t)^2$)	1
c_4	Weight per proportion reduction squared ($u(t)^2$) in birth rate	1
c_5	Weight per proportion reduction squared (${\omega_1}^2$) in the shape parameter for self-regulation (α)	10
c_6	Weight per proportion reduction squared (${\omega_2}^2$) in leptospire lifespan	10

The weight c_1, associated with a rat of any age class or infection status, can be thought of as equivalent to a proportion of the cost of human infection, assuming that any rat has the potential to infect a human in its lifetime. The relationship between the number of rats and the risk of human infection is not well-understood, and so we assume a linear relationship between the "weight" of a rat and the number of rats, specified in the objective function in equation (8) as $c_1 H(t)$. We include quadratic terms for the control measures to account for the non-linear costs at high levels of control (**Table 2**) (Miller Neilan et al., 2010; Posny et al., 2015; Malik et al., 2016).

We investigate the optimal controls under the assumption that rodenticide control has the same weight (cost) per unit as resource reduction, and that the permanent environment controls are 10 times more expensive, but that both environment controls have the same weight (**Table 2**). We assigned weights to each rat and to each log10 leptospires in the environment. We assumed that if one rat had a rate of 1, then each log10 of leptospires had a relative weight of 0.1. Infected rats are the source of leptospires in the environment, are a pest species and once infected, shed for their entire lifetime. Hence, we assigned a higher weight to rats because we anticipate that rodent control well-received and potentially more effective.

The optimal controls are found by solving the age-structured model forward in time using initial values for the control measures. Then the adjoint equations are solved backward in time using the solutions of the age-structured model. The values of the control measures are then updated using Equations (9, 10). This process is repeated until the level of the control measures have converged. The convergence criterion used was that the values from subsequent iterations were the same to five decimal places.

We assume that the time-dependent control measures, rodenticide, and resource reduction, would at most be applied for a continuous period of 30 days (Pertile et al., unpublished; de Masi et al., 2009). Hence, we investigate the optimal temporary rodent controls (rodenticide and resource reduction) for a period of 30 days. In our study system, rodent population sizes remain relatively constant throughout the year (Panti-May et al., 2016), and so we did not investigate different timings of the controls throughout the year. We also find the optimal temporary controls assuming environmental controls had been applied 30 days beforehand. Given the optimal controls, the age structured model

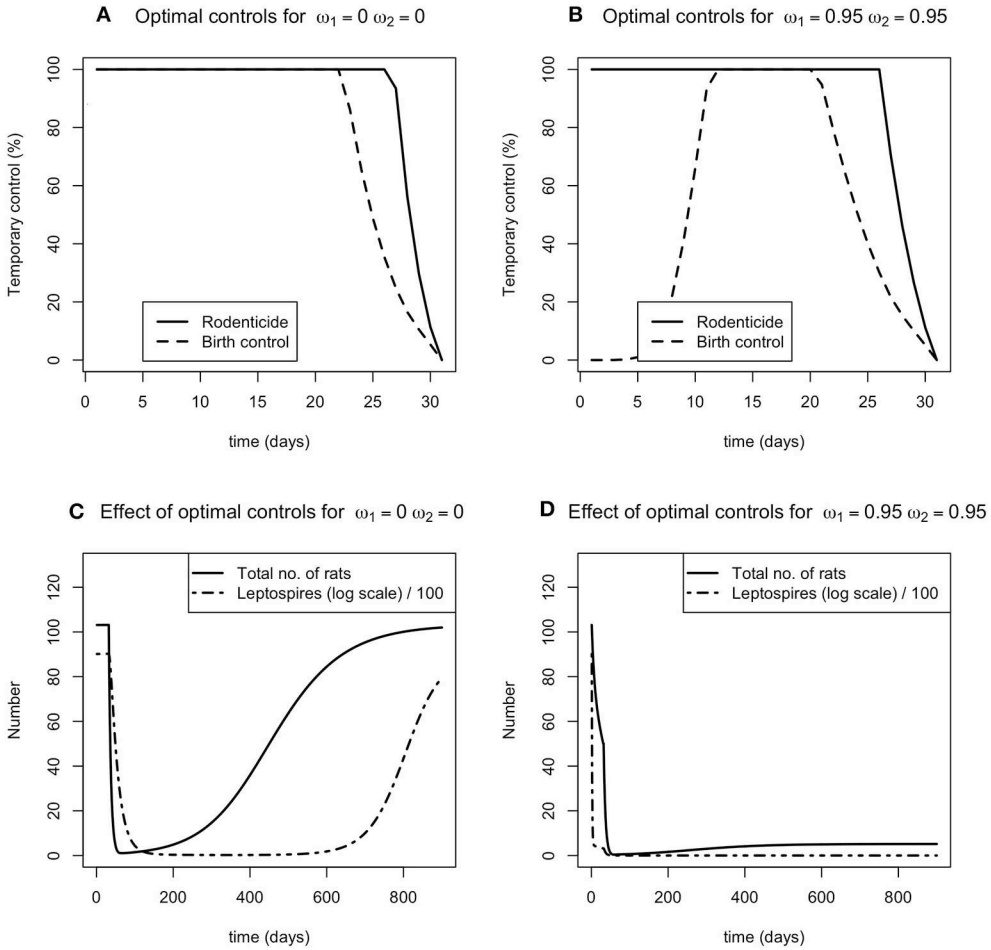

FIGURE 2 | (A,B) The optimal level of the time dependent controls, rodenticide (solid line) and resource reduction (dashed line), when the environmental controls are a) not applied ($\omega_1 = 0, \omega_2 = 0$), **(B)** applied at a high level ($\omega_1 = 0.95, \omega_2 = 0.95$). **(C,D)** The effect of no and high control (**C,D**, respectively) on the total number of rats (solid line) and number of leptospires (dot dashed line).

with control (Equations 1–8) was applied for the control period (60 days = 30 days permanent control effect +30 days of temporary control measures applied) and a period of no control (540 days) to investigate the longer term effect of these controls on infection dynamics. As measures of success of the control scenarios, we calculated the cumulative total number of rats, cumulative number of leptospires and the total weight of the different scenarios. Finally, given the uncertainties surrounding the weights assigned to the different controls, we present a sensitivity analysis of these weights.

RESULTS

For contrasting levels (none and high) of the two permanent environmental controls, carrying capacity control and leptospire mortality control, there was no distinguishable difference in the optimal level of rodenticide application in a 30 day period (**Figures 2A,B**). For most of the control period, 100% of the rodent population needs to be targeted with rodenticide in order to reduce the total rodent population and the number of free living leptospires (**Figures 2A,B**). For resource reduction, when there is no additional permanent control, the birth rate should be reduced by 100% for most of the control period, though for a slightly shorter period than rodenticide (**Figure 2A**). However, as the reduction in carrying capacity, ω_1 increases, the optimal level of resource reduction is 0 in the first few days of the control program (**Figure 2B**).

These permanent controls reduce the number of infected rats and leptospires to new lower endemic equilibria. When the reduction in carrying capacity is at its highest, there is a sharp reduction in the number of rats in the population (**Figures 2C,D**). The temporary controls alone reduce the number of infected rats and leptospires to a low level for fixed period of time, following which the rodent population recovers and reaches its previous carrying capacity, accompanied by a delayed response from the number of leptospires.

The cumulative number of leptospires over the control and no control period was reduced both by high reductions in the

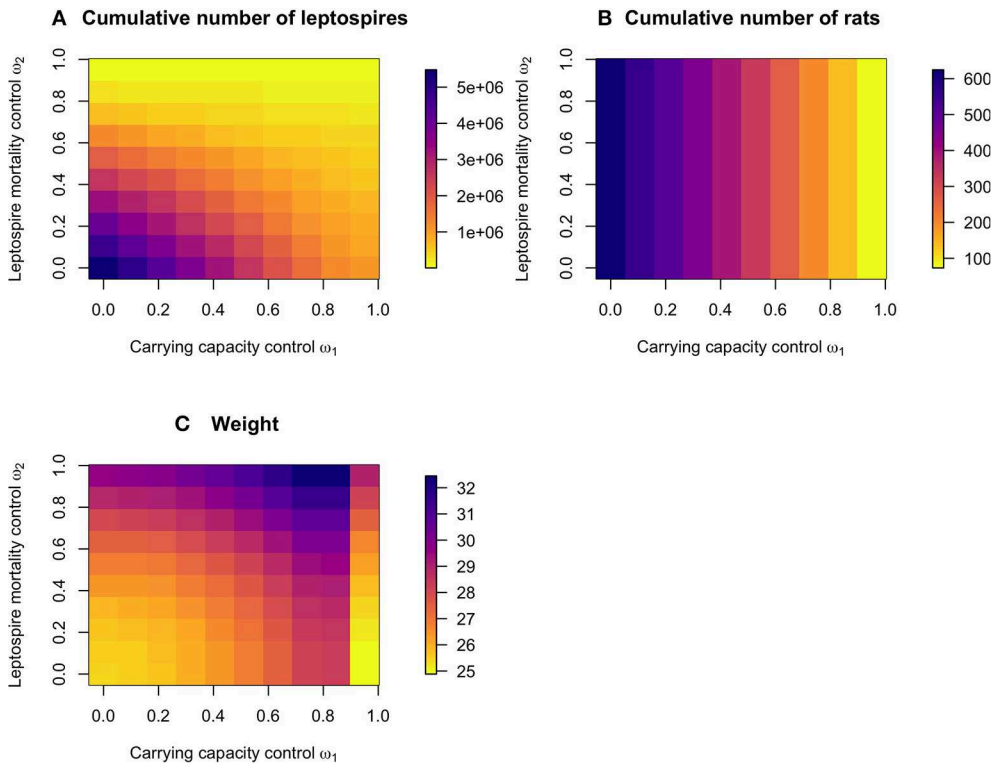

FIGURE 3 | The predicted effect of the permanent and temporary controls on **(A)** the cumulative number of leptospires on the log10 scale, and **(B)** the cumulative number of rats over the 600 days period, with **(C)** the associated cumulative weights of the control measures.

carrying capacity and the leptospire mortality rate (**Figure 3A**). The cumulative number of rats, however, was only reduced only by the carrying capacity control (**Figure 3B**). The associated weights of the control scenarios were lowest when low levels of either of the permanent control were applied, but also when a very high level of carrying capacity reduction was applied (**Figure 3C**), since a high reduction in carrying capacity requires very little subsequent application of resource reduction (as also illustrated in **Figure 2**).

The optimal controls of resource reduction and rodenticide were differentially sensitive to changes in the weights (**Figure 4**). When the resource reduction had a higher weight, the optimal level of resource reduction was much reduced, whereas the optimal level of rodenticide application was little affected (**Figure 4B**). The sensitivity was less to changes in rodenticide weights (**Figures 4C,D**). Higher weights led to a somewhat reduced period over which 100% control was optimal, but to little change in the optima for resource reduction.

DISCUSSION

Human zoonotic infections can be prevented by reducing the size of the reservoir population maintaining a pathogen, which, as in the case of leptospirosis, may also reduce the environmental load of pathogens, resulting in reduced intra- and inter-specific transmission. We present a framework to help identify and explore empirically an optimal mix of control measures to reduce the risk of human infection with leptospires. Application of temporary control measures following permanent changes to the environment is effective in reducing the rodent population size and the number of leptospires to a low level.

Permanently reducing the overall level of risk of exposure and infection among rats and humans would be effective in preventing human contact with open-sewer/contaminated water soil. Permanent controls have the added benefit of reducing risk of other diseases, especially diarrheal infections, and upgrading the urban slum environment. However, risk reduction may not be decreased sufficiently to significantly reduce cases of human leptospirosis during high risk periods, such as rainy seasons (Hagan et al., 2016). Though the temporary rodent controls result in an eventual return to the initial carrying capacity, the immediate effect of the controls could create a significant reduction in leptospires when humans are at highest risk of infection.

Control by rodenticide alone is never effective for Norway rat eradication as bait placement will not reach all rats and often, as in Salvador, rodenticide use is reactive, and placement is often focused around residents from which incident human leptospirosis cases have been recently identified. To

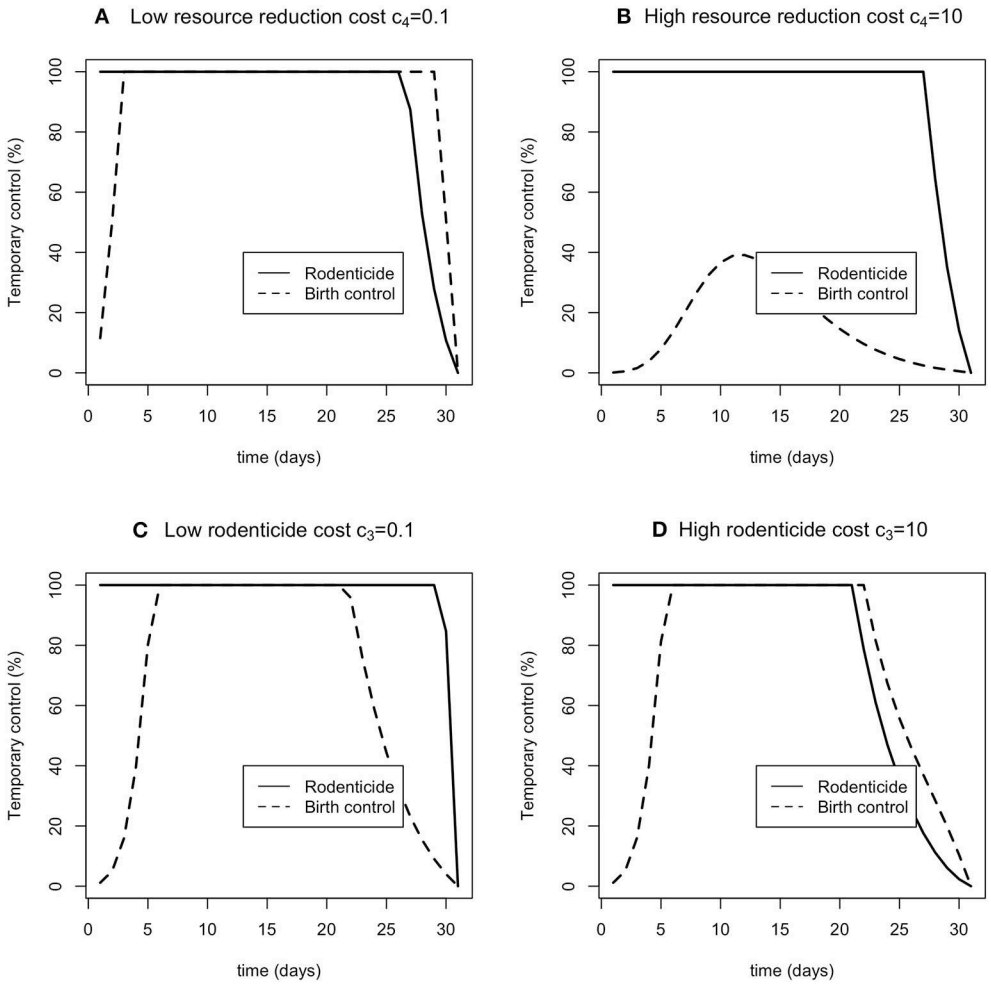

FIGURE 4 | The sensitivity of the optimal time dependent controls rodenticide (solid line) and resource reduction (dashed line) for low and high costs of rodenticide **(A,B)** and resource reduction **(C,D)**.

further complicate this control, owner permission is required prior to placement, and when an adult home owner is unavailable at the first attempt follow-up attempts may not occur. Additionally, the neophobic behavior of rats ensures remnant populations remain, which is regarded as a major barrier to the success of rodenticide campaigns (Clapperton, 2006; Feng and Himsworth, 2014). Moreover, it is logistically difficult to design a rodenticide campaign targeting a pre-defined proportion of the rodent population, since consistently accessing the required number of households to apply rodenticide is difficult.

Habitat management reduces survival by eliminating refuges (Lambert et al., 2008; Buckle, 2013), but should be extensive enough to sufficiently cover the "typical" home range of rats. The home or activity range of urban rats is small: in the order of 10 s of meters in temperate urban locations (Feng and Himsworth, 2014) in contrast to rats inhabiting rural areas (Lambert et al., 2008). For rodents in urban settings, clearing garbage will reduce food, and in some cases, refuge sources, but data on empirical effects are unavailable and require pilot field studies (see below). In some cases, to clear garbage and other solid waste, there needs to be improvements in infrastructure, such as construction of roads and identifying local and city-level recycling centers that can take in the refuse. This complicates the logistics and cost calculations for habitat management interventions.

To that end, further complexities of the formulation of the control measures should be explored such as time dependent effectiveness of rodenticide and rodent behavior in response to population changes. Though death can be assumed upon ingestion of sufficient rodenticide, death does not occur instantaneously and takes up to 8 days following consumption and dose acquired (Mlynarèíková et al., 1999). This time lag may also apply to resource reduction (Williams, 2007) such as the removal of garbage influencing the population size and birth rates. Additionally, we assumed that juveniles would not be affected by application of rodenticide. Norway rats adopt communal nursing behavior, which leads to better survival

of abandoned young, and these young, whose parents have been killed via rodenticide, may thereby survive (Meaney and Stewart, 1981; Butler and Whelan, 1994). If the population size becomes low enough, however, this nursing behavior cannot occur (Hein and Jacob, 2015), and it is expected that those animals in the nest will die as a result of a rodenticide campaign. This population size-dependent behavior has not been included in the modeling framework, which could lead to an underestimation of the effectiveness of rodenticide control.

Our model parameters were chosen to accurately predict leptospiral carriage prevalence among sub-adult and adult populations. However, model predictions of the effects of control measures have not been validated. To use this framework to plan control measures, the model framework for predicting prevalence should be validated using data from both successful and unsuccessful interventions (Joseph et al., 2013). Our analysis explored multiple control scenarios with different weights (costs), but the costs of the different controls, set against the cost of the existence of rats and leptospires in the environment, are not known. Estimating such costs is a priority.

AUTHOR CONTRIBUTIONS

AM carried out the analyses and created the figures. All authors contributed to writing and editing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00209/full#supplementary-material

REFERENCES

Adrichem, M. H. C., Van Buijs, J. A., Goedhart, P. W., and Verboom, J. (2013). Factors influencing the density of the brown rat (*Rattus norvegicus*) in and around Houses In- Amsterdam. *J. Dutch Mamm. Soc.* 56, 77–91.

Ashford, D. A., David, J. R., Freire, M., David, R., Sherlock, I., Eulálio, M. C., et al., (1998). Studies on control of visceral leishmaniasis: impact of dog control on canine and human visceral leishmaniasis in Jacobina, Bahia, Brazil. *Am. J. Trop. Med. Hyg.* 59, 53–57. doi: 10.4269/ajtmh.1998.59.53

Buckle, A. (2013). Anticoagulant resistance in the United Kingdom and a new guideline for the management of resistant infestations of norway rats (*Rattus norvegicus* Berk.). *Pest Manag. Sci.* 69, 334–341. doi: 10.1002/ps.3309

Butler, F. T., and Whelan, J. (1994). Population-structure and reproduction in brown-rats (*Rattus norvegicus*) from Pig Farms, Co Kildare, Ireland. *J. Zool.* 233, 277–291. doi: 10.1111/j.1469-7998.1994.tb08588.x

Calhoun, J. B. (1962). *The Ecology and Sociology of the Norway Rat (US Public Health Service Publication No. 1008)*. Washington, DC: US Government Printing Office.

Casanovas-Massanaa, A., Pedra, G. G., Wunder E. A. Jr., Diggle, P. J., Begon, M., and Albert I, (2018). Quantitative survival of leptospira interrogans in soil and water microcosms. *Am. Soc. Microbiol.* 84, e00507–e00518. doi: 10.1128/AEM.00507-18

Clapperton, B. K. (2006). *A Review of the Current Knowledge of Rodent Behavior in Relation to Control Devices*. Wellington, NZ: Science & Technical Publishing Deptartment of Conservation, 1–55.

Costa, F., Carvalho-Pereira, T., Begon, M., Riley, L., and Childs, J. (2017). Zoonotic and vector-borne diseases in urban slums: opportunities for intervention. *Trends Parasitol.* 33, 660–662. doi: 10.1016/j.pt.2017.05.010

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., et al., (2015a). Global morbidity and mortality of leptospirosis: a systematic review. *PLOS Negl. Trop. Dis.* 9:e0003898. doi: 10.1371/journal.pntd.0003898

Costa, F., Porter, F. H., Rodrigues, G., Farias, H., de Faria, M. T., Wunder, E., et al., (2014a). Infections by leptospira interrogans, seoul virus, and Bartonella Spp. among Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil. *Vector Borne Zoon. Dis.* 14, 33–40. doi: 10.1089/vbz.2013.1378

Costa, F., Ribeiro, G. S., Felzemburgh, R. D. M., Santos, N., Reis, R. B., Santos, A. C., et al., (2014b). Influence of household rat infestation on leptospira transmission in the urban slum environment. *PLoS Negl. Trop.Dis.* 8:e3338. doi: 10.1371/journal.pntd.0003338

Costa, F., Wunder, E., De Oliveira, D., Bisht, V., Rodrigues, G., Reis M. G., et al., (2015b). Patterns in leptospira shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission. *PLOS Negl. Trop. Dis.* 9:e0003819. doi: 10.1371/journal.pntd.0003819

de Masi, E., Vilaça, P. J., and Razzolini, M. T. P. (2009). Evaluation on the effectiveness of actions for controlling infestation by rodents in Campo Limpo Region, São Paulo municipality, Brazil. *Int. J. Environ. Health Res.* 19, 291–304. doi: 10.1080/09603120802592723

De Oliveira, D., Figueira, C. P., Zhan, L., Pertile, A. C., Pedra, G. G., Gusmão, I. M., et al., (2016). Leptospira in breast tissue and milk of urban norway rats (*Rattus norvegicus*). *Epidemiol. Infect.* 144, 1–10. doi: 10.1017/S0950268816000637

Ellis, W. A. (2015). "Animal leptospirosis," in *Leptospira and Leptospirosis*, ed B. Ellis-Adler (Berlin; Heidelberg: Springer), 99–137. doi: 10.1007/978-3-662-45059-8_6

Evangelista, K. V., and Coburn, J. (2011). Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Fut. Microbiol.* 5, 1413–1425. doi: 10.2217/fmb.10.102

Feng, A. Y. T., and Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban norway and black rats (*Rattus norvegicus* and Rattus Rattus). *Urban Ecosyst.* 17, 149–162. doi: 10.1007/s11252-013-0305-4

Galef, B. G. (1981). "The ecology of weaning," in *Parental Care in Mammals*, eds D. J. Gubernick and P. H. Klopfer (Boston, MA: Springer US), 211–241. doi: 10.1007/978-1-4613-3150-6_6

Glass, G., Childs, J., Korch, G., and LeDuc, J. (1989). *Comparative Ecology and Social Interactions of Norway Rat (Rattus norvegicus) Populations in Baltimore, Maryland*. Occasional Papers of the Museum of Natural History. The University of Kansas, 1–33.

Hagan, J. E., Moraga, P., Costa, F., Capian, N., Ribeiro, G. S., Wunder, E. A., et al., (2016). Spatiotemporal determinants of urban leptospirosis transmission: four-year prospective cohort study of slum residents in Brazil. *PLoS Negl. Trop. Dis.* 10:e0004275. doi: 10.1371/journal.pntd.0004275

Hein, S., and Jacob, J. (2015). Recovery of small rodent populations after population collapse. *Wildlife Res.* 42, 108–118. doi: 10.1071/WR14165

Hethcote, H. (2000). The mathematics of infectious diseases. *SIAM Rev.* 42, 599–653. doi: 10.1137/S0036144500371907

Holt, J., Davis, S., and Leirs, H. (2006). A Model of leptospirosis infection in an african rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. *Acta Trop.* 99, 218–225. doi: 10.1016/j.actatropica.2006.08.003

Joseph, M. B., Mihaljevic, J. R., Arellano, A. L., Kueneman, J. G., Preston, D. L., Cross, P. C., et al. (2013). Taming wildlife disease: bridging the gap between science and management. *J. Appl. Ecol.* 50, 702–712. doi: 10.1111/1365-2664.12084

Lambert, M. S., Quy, R. J., Smith, R. H., and Cowan, D. P. (2008). The effect of habitat management on home-range size and survival of rural Norway rat populations. *J. Appl. Ecol.* 45, 1753–1761. doi: 10.1111/j.1365-2664.2008.01543.x

Malik, T., Imran, M., and Jayaraman, R. (2016). Optimal control with multiple human papillomavirus vaccines. *J. Theor. Biol.* 393, 179–193. doi: 10.1016/j.jtbi.2016.01.004

Meaney, M. J., and Stewart, J. (1981). A descriptive study of social development in the rat (*Rattus norvegicus*). *Anim. Behav.* 29, 34–45. doi: 10.1016/S0003-3472(81)80149-2

Miller Neilan, R. L., Schaefer, E., Gaff, H., Fister, K. R., and Lenhart, S. (2010). Modeling optimal intervention strategies for cholera. *Bull. Math. Biol.* 72, 2004–18. doi: 10.1007/s11538-010-9521-8

Minter, A., Diggle, P. J., Costa, F., Childs, J., Ko, A. I., and Begon, M. (2017). Evidence of multiple intraspecific transmission routes for leptospira acquisition in Norway rats (*Rattus norvegicus*). *Epidemiol. Infect.* 145, 3438–3448. doi: 10.1017/S0950268817002539

Minter, A., Diggle, P. J., Costa, F., Childs, J., Ko, A. I., and Begon, M. (2018). A model for leptospire dynamics and control in the Norway rat (*Rattus norvegicus*) the reservoir host in urban slum environments. *Epidemics* 25, 26–34. doi: 10.1016/j.epidem.2018.05.002

Mlynarèíková, H., Legáth, J., Molnár, L., and Kovalkovièová, N. (1999). "Study of effectiveness of anticoagulant rodenticides used in Slovak republic," *in Proceedings of 3rd Int Conf–Urban Pests, Prague,* eds W. M. H. Robinson, F. Rettich, and G. W. Rambo (Hronov: Grafické Závody).

Panti-May, J. A., Carvalho-Pereira, T. S. A., Serrano, S., Pedra, G. G., Taylor, J., Pertile, A. C., et al., (2016). A two-year ecological study of Norway rats (*Rattus norvegicus*) in a Brazilian urban slum. *PLoS ONE* 11:e0152511. doi: 10.1371/journal.pone.0152511

Posny, D., Wang, J., Mukandavire, Z., and Modnak, C. (2015). Analyzing transmission dynamics of cholera with public health interventions. *Math. Biosci.* 264, 38–53. doi: 10.1016/j.mbs.2015.03.006

Reis, R. B., Ribeiro, G. S., Felzemburgh, R. D. M., Santana, F. S., Mohr, S., Melendez, A. X. T. O., et al. (2008). Impact of environment and social gradient on leptospira infection in urban slums. *PLoS Negl. Trop. Dis.* 2:e228. doi: 10.1371/journal.pntd.0000228

Sharomi, O., and Malik, T. (2015). Optimal control in epidemiology. *Ann. Oper. Res.* 251, 55–71. doi: 10.1007/s10479-015-1834-4

Shilova, S. A., and Tchabovsky, A. V. (2009). Population response of rodents to control with rodenticides. *Curr. Zool.* 55, 81–91

Traweger, D., Travnitzky, R., Moser, C., Walzer, C., and Bernatzky, G. (2006). Habitat preferences and distribution of the brown rat (*Rattus norvegicus* Berk.) in the city of Salzburg (Austria): implications for an urban rat management. *J. Pest Sci.* 79, 113–125. doi: 10.1007/s10340-006-0123-z

Villafañe, I. E. G., Cavia, R., Victoria Vadell, M., Suárez, O. V., and Busch, M. (2013). Differences in Population parameters of *Rattus norvegicus* in urban and rural habitats of central Argentina. *Mammalia* 77, 187–193. doi: 10.1515/mammalia-2012-0075

Williams, B. K. (2007). Optimal management of non-Markovian biological populations. *Ecol. Model.* 200, 234–242. doi: 10.1016/j.ecolmodel.2006.07.039

Zhang, Y.-Z., Zou, Y., Fu, Z. F., and Plyusnin, A. (2010). Hantavirus infections in humans and animals, China. *Emerg. Infect. Dis.* 16, 1195–1203. doi: 10.3201/eid1608.090470

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