ECOLOGY AND EVOLUTION OF NON-CONSUMPTIVE EFFECTS IN HOST-PARASITE INTERACTIONS

EDITED BY: Lien Luong, Julia Buck, Janet Koprivnikar and Sara B. Weinstein
PUBLISHED IN: Frontiers in Ecology and Evolution

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-913-6
DOI 10.3389/978-2-88971-913-6

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

ECOLOGY AND EVOLUTION OF NON-CONSUMPTIVE EFFECTS IN HOST-PARASITE INTERACTIONS

Topic Editors:
Lien Luong, University of Alberta, Canada
Julia Buck, University of North Carolina at Wilmington, United States
Janet Koprivnikar, Ryerson University, Canada
Sara B. Weinstein, The University of Utah, United States

Citation: Luong, L., Buck, J., Koprivnikar, J., Weinstein, S. B., eds. (2021). Ecology and Evolution of Non-Consumptive Effects in Host-Parasite Interactions. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-913-6

Table of Contents

frontiers
in Ecology and Evolution

EDITORIAL
published: 29 October 2021
doi: 10.3389/fevo.2021.779102

Editorial: Ecology and Evolution of Non-Consumptive Effects in Host-Parasite Interactions

Janet Koprivnikar[1], Sara B. Weinstein[2], Lien T. Luong[3] and Julia C. Buck[4]*

[1] *Department of Chemistry and Biology, Ryerson University, Toronto, ON, Canada,* [2] *School of Biological Sciences, University of Utah, Salt Lake City, UT, United States,* [3] *Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada,* [4] *Department of Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, NC, United States*

Keywords: avoidance, natural enemy, parasite, non-consumptive effect, risk, infection

Editorial on the Research Topic

Ecology and Evolution of Non-Consumptive Effects in Host-Parasite Interactions

OPEN ACCESS

Edited and reviewed by:
Jordi Figuerola,
Estación Biológica de Doñana (EBD),
Spain

****Correspondence:***
Janet Koprivnikar
jkoprivn@ryerson.ca

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *17 September 2021*
Accepted: *11 October 2021*
Published: *29 October 2021*

Citation:
Koprivnikar J, Weinstein SB, Luong LT and Buck JC (2021) Editorial: Ecology and Evolution of Non-Consumptive Effects in Host-Parasite Interactions. Front. Ecol. Evol. 9:779102. doi: 10.3389/fevo.2021.779102

INTRODUCTION

The COVID-19 pandemic has vividly illustrated that when a highly contagious, deadly pathogen begins circulating, humans adjust their behavior to reduce their risk of contracting the disease. Such avoidance-induced changes in social interactions, movement, and food acquisition, i.e., "non-consumptive effects" (NCEs), can be extremely costly to hosts, reducing our GDP, impacting our mental and physical health, and shrinking our birth rates (e.g., Buck and Weinstein, 2020). Furthermore, infection avoidance can trigger cascading effects on other species and the environment. For instance, lockdowns reduced greenhouse gas emissions and caused behavioral changes in various animal species (Bates et al., 2021; Montgomery et al., 2021). But humans are not the only species that experiences infection outbreaks. Animals, too, host a variety of infectious agents, and behaviorally-mediated parasite avoidance is increasingly recognized as widespread and important (e.g., Behringer et al., 2018; Hart and Hart, 2018).

This special issue draws attention to the mounting evidence that parasites and pathogens can impose NCEs on hosts, and the diversity of study systems spanned by the contributed articles speak to the generality of this phenomenon. These provide examples of behavioral mitigation of infection risk by various animals, from mollusks to mammals, as well as considering the consequences of behavioral trait changes, and the evolution of strategies to avoid infection. The inclusion of different host-parasite systems adds considerable phylogenetic breadth to our current state of knowledge—-a critical element as we work toward identifying and quantifying the effects exerted by natural enemies irrespective of how they consume their victim (infection in this case). Both invertebrate and vertebrate hosts are investigated, with parasites ranging from helminths and fungi to biting flies, as well as aquatic and terrestrial habitats. The findings of the contributors clearly demonstrate that different animal species engage in behavioral avoidance to reduce their infection risk, and that this can take different forms. In addition, this Research Topic takes a comprehensive view in

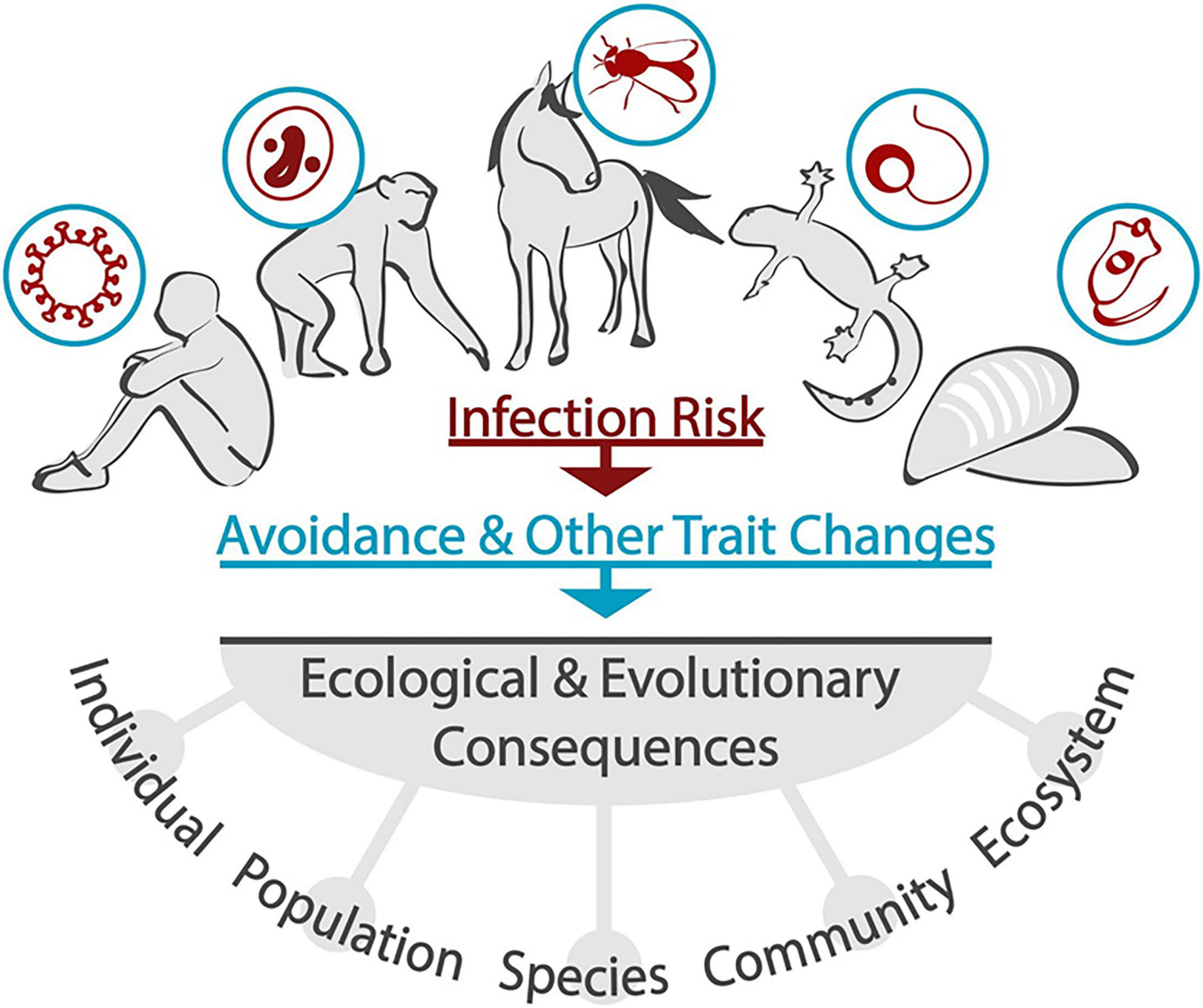

FIGURE 1 | Across diverse hosts and parasites, infection risk generates avoidance *via* changes in behavior and other traits. Papers in this special topic provide insight into the mechanisms of detection, the range of avoidance behaviors, trade-offs associated with reducing infection risk, and the ecological and evolutionary consequences of avoiding infection. As demonstrated by Selbach and Mouritsen, consequences can occur at a range of scales, from individual (e.g., reduced feeding) to ecosystem (e.g., altered nutrient cycling). Effects at the individual and population levels can also exert selective pressure, acting as feedback on avoidance behaviors to increase or maintain these for any given host-parasite system.

considering the occurrence and consequences of parasite/pathogen-induced NCEs from beginning to end. This starts with detection of risk by potential victims, followed by avoidance behaviors, and some of these studies are the first to show that parasite-induced NCEs can affect host populations, community interactions, and ecosystem structures (**Figure 1**). Such an integrated viewpoint is necessary to better understand when and why host trait alterations occur in response to the threat posed by parasites and pathogens. As the NCEs of parasites have been largely ignored until relatively recently, we may be severely underestimating the total cost of living in an infectious world. Below we briefly summarize the key messages of the contributed articles, followed by suggestions for future studies that build upon these.

BEHAVIORAL AVOIDANCE OF INFECTION

Lopes broadly observes that social-distancing measures employed during the COVID-19 pandemic correspond to strategies used by non-human animals, and that we are not alone in using such tactics to reduce infection risk. To this end, Friesen and Detwiler explore the means by which potential hosts detect and avoid transmission risk, particularly in aquatic environments, where potential hosts may use chemical cues (oxylipins) to detect parasitized conspecifics and avoid becoming infected. Working at the interface between aquatic and terrestrial environments, Daversa et al. demonstrate that alpine newts use non-visual cues from parasite-exposed conspecifics to inform habitat avoidance that could reduce their risk of

Batrachochytrium dendrobatidis infection. However, these newts might experience NCEs as a result of altered habitat use. Sarabian et al. find that bonobos avoid soil- and fecally-contaminated food, with individuals exhibiting the strongest avoidance behaviors the least likely to be infected by *Balantioides coli*, a parasitic protozoan with an oral-fecal route of transmission. Rubenstein and Feinstein show that horses adjust their daily habitat use to avoid being bitten by blood-drinking flies, basing foraging decisions on the speed of winds that mitigate this risk. As different habitats provide different nutrients, here, parasite avoidance might not require the trade-offs often seen in other systems.

CONSEQUENCES OF PARASITE/PATHOGEN AVOIDANCE

Selbach and Mouritsen consider how reductions in mussel filtering activity to avoid trematode infection might have far-reaching consequences for aquatic communities, with parasites acting as cryptic ecosystem engineers via NCEs. Koprivnikar et al. examine similarities and differences between host-parasite and plant-invertebrate herbivore systems in terms of natural enemy detection and potential for risk-induced trait alterations. Such comparisons may prove useful for predicting the occurrence and costs of NCEs.

EVOLUTION OF INFECTION AVOIDANCE STRATEGIES

Amoroso evaluates whether physiological resistance to infection is a useful framework for considering the evolution of behavioral resistance, concluding that there are some benefits, with avoidance behaviors more likely to represent an innate rather than a learned strategy. Poulin et al. develop a "ghost of parasitism past" hypothesis, suggesting that animal species with lower than expected parasite diversity are a good starting point in the search for traces of past parasite-mediated selection. This could help explain the dynamic and inconsistent relationship between the expression of avoidance traits and relative infection risk in comparative analyses across host species. Relatedly, Doherty and Ruehle consider the evolution of avoidance behaviors in an integrated "landscape of peril" containing predators, parasites, and other natural enemies that may exert distinct selective pressures.

CONCLUSIONS

Articles contributed to this Research Topic have advanced our knowledge of the ecology and evolution of NCEs, but clearly some gaps persist. Specifically, examples of risk-induced trait alterations from a broader range of host and parasite species are needed. The emphasis to date has been on behavioral alterations, but hosts could also alter other traits (e.g., morphology, physiology, development) to avoid parasites. Because the fitness costs of trait alterations are often assumed rather than directly measured (e.g., Luong et al., 2017), we still have little understanding of how consumptive and non-consumptive effects of parasites compare in terms of frequency and cost. Although a few trait-mediated effects triggered by parasite NCEs have been reported (including in this Research Topic), these pale in comparison to density- and trait-mediated indirect effects stemming from consumption by parasites (Buck, 2019). Furthermore, consequences for ecosystem structure and function remain largely unknown, although it has been shown that predators can elicit such effects. Given that NCEs are ubiquitous yet often overlooked, the true cost of parasitism may be underestimated in many host populations, making this an important area of continued study.

AUTHOR CONTRIBUTIONS

JK, SW, LL, and JB wrote and edited the article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to JK (04622), and the Ruth L. Kirschstein National Research Service Award to SW (NIH T32AI055434).

ACKNOWLEDGMENTS

We thank the editorial board at Frontiers in Ecology and Evolution for their support, as well as the many peer reviewers of the contributed articles.

REFERENCES

Bates, A. E., Primack, R. B., Duarte, C. M., and PAN-Environment Working Group (2021). Global COVID-19 lockdown highlights humans as both threats and custodians of the environment. *Biol. Conserv.* 20:109175. doi: 10.1016/j.biocon.2021.109175

Behringer, D. C., Karvonen, A., and Bojko, J. (2018). Parasite avoidance behaviors in aquatic environments. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170202. doi: 10.1098/rstb.2017.0202

Buck, J. C. (2019). Indirect effects explain the role of parasites in ecosystems. *Trends Parasitol.* 35, 835–847. doi: 10.1016/j.pt.2019.07.007

Buck, J. C., and Weinstein, S. B. (2020). The ecological consequences of a pandemic. *Biol. Lett.* 6:20200641. doi: 10.1098/rsbl.2020.0641

Hart, B. L., and Hart, L. A. (2018). How mammals stay healthy in nature: the evolution of behaviors to avoid parasites and pathogens. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170205. doi: 10.1098/rstb.2017.0205

Luong, L. T., Horn, C. J., and Brophy, T. (2017). Mitey costly: energetic costs of parasite avoidance and infection. *Physiol. Biochem. Zool.* 90, 471–477. doi: 10.1086/691704

Montgomery, R. A., Raupp, J., and Parkhurst, M. (2021). Animal behavioral responses to the COVID-19 quietus. *Trends Ecol. Evol.* 36, 184–186. doi: 10.1016/j.tree.2020.12.008

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

OPINION
published: 16 June 2020
doi: 10.3389/fevo.2020.00172

We Are Not Alone in Trying to Be Alone

*Patricia C. Lopes**

Schmid College of Science and Technology, Chapman University, Orange, CA, United States

Keywords: COVID-19, social behavior, sickness behavior, parasite avoidance, disgust avoidance, disease transmission

Certain diseases, like colds, tend not to stop us. A paracetamol here, an ibuprofen there, and we are on the go. That is, until we, as a species, are faced with a virus that not only spreads through social contact, but has an estimated reproductive number of 2 to 2.5 and potentially kills 3–4% of those infected (WHO, 2020). To reduce transmission probability of COVID-19, governmental agencies around the world have recommended or enforced measures to decrease social contact; early evidence suggests these measures produce the intended effect (Kucharski et al., 2020).

WOULD WE HUMANS TEND TO DO THIS NATURALLY?

Interesting insights can be found from studying how other animal species change their behavior when infectious disease is present, and by considering the extent to which these changes are self-regulated or enforced by other individuals. Recently, a study by Stockmaier et al. (2020) suggested that vampire bats (*Desmondus rotundus*) decrease social contacts when exposed to an immune challenge, but in a non-random way. Contacts between mothers and their offspring are maintained regardless of either being immune challenged but contacts of immune challenged animals to non-close kin are decreased. In this instance, the behavior seems to be self-regulated. Wild mice (*Mus domesticus*) have also been found to cut ties to their social groups when feeling sick (Lopes et al., 2016), but in this case kinship does not appear to play a role (Lopes et al., 2018). The effects of sickness in reducing host social contacts tend to be so robust that, in laboratory rodent studies, a standard test to verify sickness symptoms quantifies the decrease in social exploration of juvenile conspecifics by the host (Dantzer, 2001). A group of animals that is particularly susceptible to socially transmitted parasites are social insects given the high density of individuals living together and, in many cases, the high degree of genetic similarity amongst them. Here too, it is found that pathogen-exposed individuals or even individuals dying from other, non-infectious, causes spend more time away from their colonies (Müller and Schmid-Hempel, 1993; Heinze and Walter, 2010; Bos et al., 2012; Stroeymeyt et al., 2018).

The vampire bat, rodent and social insect studies are examples of when the sick animals change their social behavior. But several studies have highlighted the ability for a number of different species to recognize disease cues and avoid animals displaying those cues. For instance, mandrills (*Mandrillus sphinx*) avoid both fecal material from and grooming of parasitized conspecifics (Poirotte et al., 2017). Female olive baboons (*Papio anubis*) behave similarly by avoiding mating with males carrying a symptomatic sexually transmitted bacterial disease (Paciência et al., 2019). Interestingly, these female baboons also avoid mating when they are themselves infected. Guppies (*Poecilia reticulata*) can use chemical and visual cues to adjust avoidance behavior of infected individuals to times when transmissibility of the parasite is the highest (Stephenson et al., 2018). Social lobsters (*Panulirus argus*) also avoid groupmates infected with a deadly virus (Behringer et al., 2006). Going back to social insects, when dampwood termites (*Zootermopsis angusticollis)* produce a vibratory display in the presence of a pathogenic fungus, nestmates distance themselves from the vibrating termites (Rosengaus et al., 1999). While detection of diseased conspecifics or

Edited by:
Patrizia d'Ettorre,
Université Paris 13, France

Reviewed by:
Dana M. Hawley,
Virginia Tech, United States
Nick Bos,
University of Helsinki, Finland

****Correspondence:***
Patricia C. Lopes
lopes@chapman.edu

Specialty section:
This article was submitted to
Social Evolution,
a section of the journal
Frontiers in Ecology and Evolution

Received: *07 April 2020*
Accepted: *14 May 2020*
Published: *16 June 2020*

Citation:
Lopes PC (2020) We Are Not Alone in
Trying to Be Alone.
Front. Ecol. Evol. 8:172.
doi: 10.3389/fevo.2020.00172

disease cues is found in social insects and can directly trigger avoidance (e.g., *Lasius niger* ant nurses, Stroeymeyt et al., 2018) or aggression (Waddington and Rothenbuhler, 1976; Drum and Rothenbuhler, 1985) in non-infected nestmates, these reactions aren't necessarily always the case (Richard et al., 2008; Leclerc and Detrain, 2016). This may be because, as explained in the previous paragraph, diseased or moribund social insects tend to show spontaneous avoidance of the colony so there is no need to develop discrimination strategies against them, and also because immunity can be socially transferred in some cases (e.g., dampwood termites, Traniello et al., 2002).

In humans, a lot of research on the pathogen avoidance topics described above for other species has been done under a framework referred to as the "behavioral immune system" (Ackerman et al., 2018; Murray et al., 2019). This term describes a system of disease detection that activates behavioral responses aimed at diminishing pathogen exposure. For instance, humans not only recognize visual cues of disease (such as a photograph of a person coughing), but they respond to those cues physiologically by priming their immune system (Schaller et al., 2010). Detection of and preference for health cues also seems to occur in humans. For example, evidence suggests that women prefer the faces and the scent of men heterozygous at Major Histocompatibility Complex (MHC) loci. Heterozygosity at MHC loci has been associated with greater resistance to certain infectious diseases in humans relative to homozygosity (reviewed in Tybur and Gangestad, 2011). Another overlapping term for this disease avoidance behavior is the "disgust adaptive system," a term sometimes used interchangeably with behavioral immune system (Curtis et al., 2011). Disgust here would be the visceral emotional reaction that generally accompanies withdrawal from people (particularly strangers) displaying cues that reliably indicate pathogen presence (Curtis et al., 2004). Although disgust is expressed universally in humans, disgust sensitivity is variable across individuals and there are currently no good hypotheses that explain this variation (Tybur et al., 2018). Disgust sensitivity seems to predict the strength of behavioral avoidance of cues of contamination (Deacon and Olatunji, 2007).

Not all disease cues, however, lead to avoidance, particularly if they are relatively novel in a population. One example in which this has been studied is conjunctivitis caused by the bacterial pathogen *Mycoplasma gallisepticum*. This is a directly transmissible pathogen that causes house finches (*Haemorhous mexicanus*) to develop visible symptoms around the eye, as well as lethargy. One study found that male house finches preferred to feed near diseased conspecifics, potentially because the diseased animals became less aggressive around food (Bouwman and Hawley, 2010). Such a result raises interesting questions regarding the extent to which animals are able to make appropriate decisions when faced with new circumstances.

Perhaps the important take-home message is that these avoidance behaviors have likely evolved because they increase survival in the presence of disease. Allowing other animals to inspire some of our social rules during this time and to teach us something about community may not be a bad idea. By adopting social distancing as part of our battle against a novel infectious disease, we are fighting against some of what it means to be human: to live socially. But simultaneously, we are also doing a tremendous act of kindness for one another and for our communities.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

Ackerman, J. M., Hill, S. E., and Murray, D. R. (2018). The behavioral immune system: current concerns and future directions. *Soc. Person. Psychol. Compass* 12:e12371. doi: 10.1111/spc3.12371

Behringer, D. C., Butler, M. J., and Shields, J. D. (2006). Avoidance of disease by social lobsters. *Nature* 441:421. doi: 10.1038/441421a

Bos, N., Lefèvre, T., Jensen, A. B., and D'ettorre, P. (2012). Sick ants become unsociable. *J. Evol. Biol.* 25, 342–351. doi: 10.1111/j.1420-9101.2011.02425.x

Bouwman, K. M., and Hawley, D. M. (2010). Sickness behaviour acting as an evolutionary trap? *Male house finches preferentially feed near diseased conspecifics. Biology Letters* 6, 462–465. doi: 10.1098/rsbl.2010.0020

Curtis, V., Aunger, R., and Rabie, T. (2004). Evidence that disgust evolved to protect from risk of disease. *Proc. R. Soc. Lond. Series B: Biol. Sci.* 271, S131–S133. doi: 10.1098/rsbl.2003.0144

Curtis, V., de Barra, M., and Aunger, R. (2011). Disgust as an adaptive system for disease avoidance behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 389–401. doi: 10.1098/rstb.2010.0117

Dantzer, R. (2001). Cytokine-induced sickness behavior: where do we stand? *Brain Behav. Immun.* 15, 7–24. doi: 10.1006/brbi.2000.0613

Deacon, B., and Olatunji, B. O. (2007). Specificity of disgust sensitivity in the prediction of behavioral avoidance in contamination fear. *Behav. Res. Therap* 45, 2110–2120. doi: 10.1016/j.brat.2007.03.008

Drum, N. H., and Rothenbuhler, W. C. (1985). Differences in non-stinging aggressive responses of worker honeybees to diseased and healthy bees in May and July. *J. Apicult. Res.* 24, 184–187. doi: 10.1080/00218839.1985.11100669

Heinze, J., and Walter, B. (2010). Moribund ants leave their nests to die in social isolation. *Curr. Biol.* 20, 249–252. doi: 10.1016/j.cub.2009.12.031

Kucharski, A. J., Russell, T. W., Diamond, C., Liu, Y., Edmunds, J., Funk, S., et al. (2020). Early dynamics of transmission and control of COVID-19: a mathematical modelling study. *Lancet Infect. Dis.* 20, 553–558. doi: 10.1016/S1473-3099(20)30144-4

Leclerc, J.-B., and Detrain, C. (2016). Ants detect but do not discriminate diseased workers within their nest. *Sci. Nat.* 103:70. doi: 10.1007/s00114-016-1394-8

Lopes, P. C., Block, P., and König, B. (2016). Infection-induced behavioural changes reduce connectivity and the potential for disease spread in wild mice contact networks. *Sci. Rep.* 6:31790. doi: 10.1038/srep31790

Lopes, P. C., Block, P., Pontiggia, A., Lindholm, A. K., and König, B. (2018). No evidence for kin protection in the expression of sickness behaviors in house mice. *Sci. Rep.* 8:16682. doi: 10.1038/s41598-018-35174-0

Müller, C. B., and Schmid-Hempel, P. (1993). Exploitation of cold temperature as defence against parasitoids in bumblebees. *Nature* 363, 65–67. doi: 10.1038/363065a0

Murray, D. R., Prokosch, M. L., and Airington, Z. (2019). Psychobehavioroimmunology: connecting the behavioral immune system to its physiological foundations. *Front. Psychol.* 10:200. doi: 10.3389/fpsyg.2019.00200

Paciência, F. M. D., Rushmore, J., Chuma, I. S., Lipende, I. F., Caillaud, D., Knauf, S., et al. (2019). Mating avoidance in female olive baboons (*Papio anubis*) infected by *Treponema pallidum*. *Sci. Adv.* 5:eaaw9724. doi: 10.1126/sciadv.aaw9724

Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., et al. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Sci. Adv.* 3:e1601721. doi: 10.1126/sciadv.1601721

Richard, F.-J., Aubert, A., and Grozinger, C. (2008). Modulation of social interactions by immune stimulation in honey bee, *Apis mellifera*, workers. *BMC Biol.* 6:50. doi: 10.1186/1741-7007-6-50

Rosengaus, R. B., Jordan, C., Lefebvre, M. L., and Traniello, J. F. A. (1999). Pathogen alarm behavior in a termite: a new form of communication in social insects. *Naturwissenschaften* 86, 544–548. doi: 10.1007/s001140050672

Schaller, M., Miller, G. E., Gervais, W. M., Yager, S., and Chen, E. (2010). Mere visual perception of other people's disease symptoms facilitates a more aggressive immune response. *Psychol. Sci,* 21, 649–652. doi: 10.1177/0956797610368064

Stephenson, J. F., Perkins, S. E., and Cable, J. (2018). Transmission risk predicts avoidance of infected conspecifics in *Trinidadian guppies*. *J. Anim. Ecol.* 87, 1525–1533. doi: 10.1111/1365-2656.12885

Stockmaier, S., Bolnick, D. I., Page, R. A., and Carter, G. G. (2020) Sickness effects on social interactions depend on the type of behaviour and relationship. *J. Anim. Ecol.*13, 1–8. doi: 10.1111/1365-2656.13193

Stroeymeyt, N., Grasse, A. V., Crespi, A., Mersch, D. P., Cremer, S., and Keller, L. (2018). Social network plasticity decreases disease transmission in a eusocial insect. *Science* 362, 941–945. doi: 10.1126/science.aat 4793

Traniello, J. F. A., Rosengaus, R. B., and Savoie, K. (2002). The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proc. Natl. Acad. Sci. U.S.A.* 99, 6838–6842. doi: 10.1073/pnas.102176599

Tybur, J. M., Çinar, Ç., Karinen, A. K., and Perone, P. (2018). Why do people vary in disgust? *Philos. Trans. R. Soc. B* 373:20170204. doi: 10.1098/rstb.2017.0204

Tybur, J. M., and Gangestad, S. W. (2011). Mate preferences and infectious disease: theoretical considerations and evidence in humans. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 3375–3388. doi: 10.1098/rstb.2011.0136

Waddington, K. D., and Rothenbuhler, W. C. (1976). Behaviour associated with hairless-black syndrome of adult honeybees. *J. Apicult. Res.* 15, 35–41. doi: 10.1080/00218839.1976.11099831

WHO (2020). *Coronavirus Disease 2019 (COVID-19)*. Situation report 46. Available online at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200306-sitrep-46-covid-19.pdf?sfvrsn=96b04adf_4 (March 6, 2020).

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

PERSPECTIVE
published: 23 June 2020
doi: 10.3389/fevo.2020.00195

Evolutionary Signature of Ancient Parasite Pressures, or the Ghost of Parasitism Past

Robert Poulin*†, Jerusha Bennett†, Daniela de Angeli Dutra†, Jean-François Doherty†, Antoine Filion†, Eunji Park† and Brandon Ruehle†

Department of Zoology, University of Otago, Dunedin, New Zealand

OPEN ACCESS

Edited by:
Lien Luong,
University of Alberta, Canada

Reviewed by:
Martin Kavaliers,
University of Western Ontario, Canada
Tommy Leung,
University of New England, Australia

***Correspondence:**
Robert Poulin
robert.poulin@otago.ac.nz

†ORCID:
Robert Poulin
orcid.org/0000-0003-1390-1206
Jerusha Bennett
orcid.org/0000-0003-1037-894X
Daniela de Angeli Dutra
orcid.org/0000-0003-2341-2035
Jean-François Doherty
orcid.org/0000-0003-4766-9417
Antoine Filion
orcid.org/0000-0003-1198-3017
Eunji Park
orcid.org/0000-0002-5087-2398
Brandon Ruehle
orcid.org/0000-0002-9898-3169

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: 09 April 2020
Accepted: 29 May 2020
Published: 23 June 2020

Citation:
Poulin R, Bennett J,
de Angeli Dutra D, Doherty J-F,
Filion A, Park E and Ruehle B (2020)
Evolutionary Signature of Ancient
Parasite Pressures, or the Ghost
of Parasitism Past.
Front. Ecol. Evol. 8:195.
doi: 10.3389/fevo.2020.00195

Animals adopt a range of avoidance strategies to reduce their exposure to parasites and the associated cost of infection. If strong selective pressures from parasites are sustained over many generations, avoidance strategies may gradually evolve from phenotypically plastic, or individually variable, to fixed, species-wide traits. Over time, host species possessing effective infection avoidance traits may lose parasite species. Indeed, if overcoming the avoidance strategies of a host species is too costly, i.e., if individuals of that species become too rarely encountered or difficult to infect, a generalist parasite may opt out of this particular arms race. From the host's perspective, if avoidance traits are not costly or have been co-opted for other functions, they may persist in extant species even if ancestral parasites are lost, as signatures of past selection by parasites. Here, we develop the "ghost of parasitism past" hypothesis. We discuss how animal species with a lower number of parasite species than expected based on their ecological properties or phylogenetic affinities are a good starting point in the search for traces of past parasite-mediated selection. We then argue that the hypothesis explains the dynamic and inconsistent nature of the relationship between the expression of avoidance traits and relative infection risk in comparative analyses across host species. Finally, we propose some approaches to test the predictions of the hypothesis. Animal morphology and behavior show clear evidence of past selective pressures from predators; we argue that past selection from parasites has also left its imprint, though in more subtle ways.

Keywords: avoidance behavior, comparative analysis, infection risk, parasite-mediated selection, parasite species richness, selection pressure

INTRODUCTION

The idea that the non-consumptive effects of predators can match or even exceed those of their direct consumption of prey has gained much evidential support in recent years (Preisser et al., 2005; Suraci et al., 2016). In particular, the fear experienced by prey and manifested as distinct behavioral or physiological responses to avoid predation can be costly, leading to reductions in reproductive output and impacts on population dynamics (Clinchy et al., 2013; Zanette and Clinchy, 2019). Similarly, the fear (or disgust) of parasites and the associated costs of infection might cause hosts to adopt particular strategies that reduce the risk of infection (Buck et al., 2018; Weinstein et al., 2018). Parasite avoidance strategies include all steps involved in preventing infection, from early detection and recognition of parasites, all the way to active evasion of infective stages. These strategies are costly, for instance by causing animals to miss out on foraging, mating or socializing opportunities (Norris, 1999; Fritzsche and Allan, 2012; Kavaliers and Choleris, 2018). As we write these lines, the threat of infection by the virus responsible for the COVID-19 disease is causing major changes worldwide to our social behavior with major repercussions on the economy, employment security, and the availability of goods and services. Who then can doubt the costs associated with the fear of infection? Despite these costs, however, avoidance strategies must confer overall net fitness gains, or else they would not be maintained.

In addition to their immediate ecological consequences, the adoption of avoidance strategies by animals and other non-consumptive effects of parasites could have evolutionary implications. Given that avoidance strategies must have overall positive fitness effects, these benefits could drive the evolution of avoidance strategies from phenotypically plastic, or individually variable, to fixed species-wide traits. For this to happen, two conditions must be met. First, the risk of infection must be high enough to represent a real danger for any individual in the population. In other words, the prevalence of infection by a costly parasite, or the diversity of parasite species that may infect a host by a particular route, must be locally high enough to lead to a high probability of incurring a cost from parasite infection. Secondly, the high infection risk must persist over time, thus exerting strong selective pressures over several generations. Situations in which both conditions are met are likely to be common (Wilson et al., 2019), and we may therefore expect some of the traits of extant animals to be the product of past selection to avoid parasite infection. These traits may not be easy to identify, however, as they may now be associated with different functions, or so ubiquitous that they are assumed to have other origins. For example, sexual reproduction is so widespread among living organisms that its potential evolutionary maintenance as a defense against fast-evolving pathogens was long overlooked (Hamilton, 1980).

Here, we discuss how strong and sustained selection of avoidance strategies against parasites in the past may have left its mark in extant species, whether or not this signature is easily detectable. In a parallel with the idea that the ghost of competition past is still visible in the character displacement and non-overlapping niches of extant species living in sympatry (Connell, 1980), we propose that the ghost of parasitism past has shaped host evolution. As examples of evolutionary anachronisms (Barlow, 2000), many behavioral, physiological or morphological traits of extant species may be ascribed to past selective pressures from parasites, i.e., pressures that are no longer measurable. We first discuss how animal species that are nowadays used by a disproportionately low number of parasite species may offer clues in the search for traces of past parasite-mediated selection. Then, we argue that the relationship between the expression of avoidance traits and relative infection risk across host species can change over evolutionary time, and thereby explain the inconsistent results from comparative analyses that have attempted to link these variables. Finally, we acknowledge the challenges of demonstrating cause-and-effect in testing the "ghost of parasitism past" hypothesis, and propose different ways to overcome these difficulties. Our focus is on long-term evolution and interspecific differences, i.e., we look at variation in avoidance strategies among different species, and how this relates to the risks of infection they now face. We consider the abundance and pathogenicity of specific parasites, as well as parasite species richness, as measures of selective pressures imposed by parasites (Bordes and Morand, 2009).

HAVE HOST SPECIES WITH FEW PARASITES EVOLVED A SOLUTION?

Have animal species with disproportionately few parasite species found the secret to parasite avoidance? Some species are exploited by fewer parasite species than what would be expected based on their ecological properties (e.g., body mass, population density, longevity, and social behavior) or phylogenetic affinities. For example, among seabirds with similar habitats, lifestyles and body sizes, members of the order Pelecaniformes (pelicans, gannets, boobies, and cormorants) consistently harbor fewer species of ectoparasitic lice than members of the orders Charadriiformes (gulls, skuas, and auks) and Procellariiformes (albatrosses and petrels) (Hughes and Page, 2007). Similarly, in anthropoid primates (monkeys and apes), even after controlling for uneven sampling effort, the species richness of helminth and protozoan parasites varied substantially among host species, with only a modest proportion of that variance explained by the combined effects of ecological variables such as body mass, population density or longevity (Nunn et al., 2003). Some primate species simply appear to be under-parasitized. Can the "ghost of parasitism past" hypothesis explain why some species have so few parasites? Do species with relatively fewer parasites possess intrinsic features that we are yet to recognize as parasite avoidance strategies? If so, what might these be?

Poulin et al. (2011b) used species-area relationship (SAR) models on several large datasets on parasite species richness in vertebrates to identify host species with more or fewer parasite species than expected based on sampling effort and body mass. Host species lying below the 80% confidence interval of the averaged SAR function were considered as hosts with disproportionately low numbers of parasites for their body mass. In most datasets, there was clear evidence of phylogenetic clustering, i.e., host species from the same family were more likely to all fall above, within or below the confidence interval of the SAR curve (Poulin et al., 2011b). Key ecological traits within a phylogenetic group also played a role. For instance, among shark species, the likelihood that a host species had a disproportionately low number of cestode parasites was influenced by the species' median depth range, although in different ways depending on the family it belonged to Poulin et al. (2011b). Depth range is indeed known to be an important determinant of cestode species richness in sharks, although depth preferences are assumed to have evolved for other reasons, and not as a strategy to avoid parasite-laden prey (Randhawa and Poulin, 2010). However, if animals perceive their environment as a landscape of risk and move preferentially to safer zones where exposure to infection

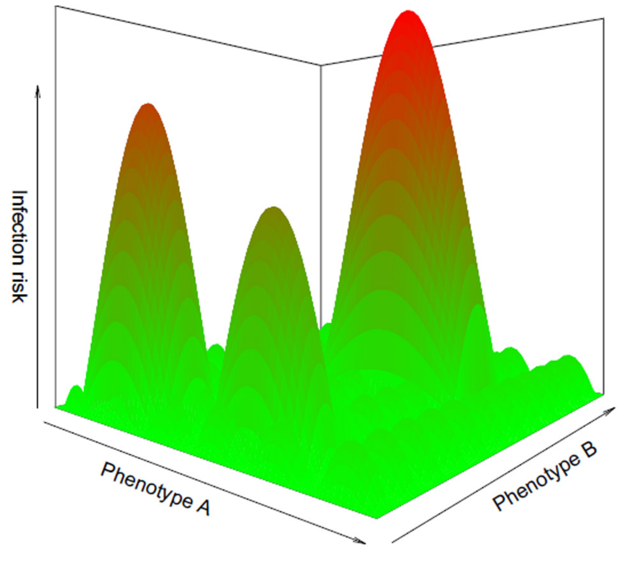

FIGURE 1 | Phenotypic landscape of infection risk, illustrating the infection risk associated with all possible combinations of values for two hypothetical phenotypic traits, A and B. Red peaks correspond to phenotypes exposing individuals to a high risk of infection, whereas green valleys correspond to safe phenotypic strategies. Species whose phenotypes settle in valleys over evolutionary time may eventually lose parasite species.

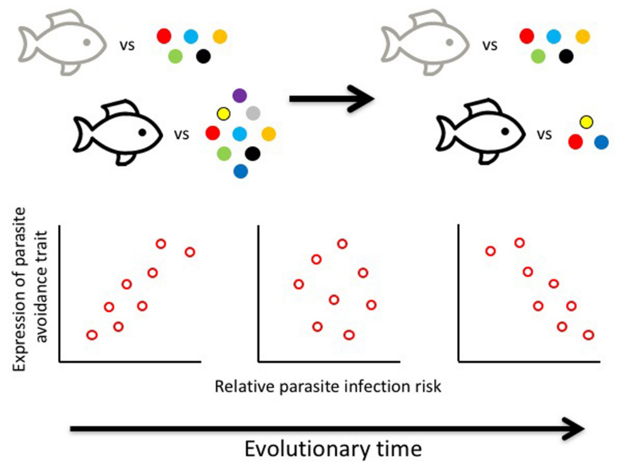

FIGURE 2 | Evolutionary dynamic relationship between infection risk and avoidance traits, as it shifts from positive to negative over evolutionary time. Species facing multiple parasite species (colored points) are under stronger selection to develop more pronounced avoidance strategies (dark shape outline) than those facing few parasites; over time, however, strong avoidance strategies may lead to the loss of parasite species. The different points on the graphs represent either distinct host species, or distinct populations of the same host species. NB: Scale on *x*-axis shows relative risk (e.g., parasite species richness), and not absolute risk; it allows comparison among points on the same graph, but not among graphs.

is lowest (Weinstein et al., 2018), we would expect some of these habitat preferences to have become fixed traits over evolutionary time.

As the physical environment represents a multi-dimensional space in which organisms occupy particular zones, diet, morphology, behavioral, and physiological traits also form continuums along which natural selection can position any given species based on net fitness benefits. If both axes of a two-dimensional space represent the full range of possible values for two behavioral traits, all potential behavioral responses to a detected infection risk can be visualized as a continuous landscape along which there are peaks, i.e., combinations of behavioral patterns corresponding to an elevated infection risk, and valleys representing safe behavioral patterns (**Figure 1**). Over time, in species selected to avoid pressures from parasites, natural selection may have narrowed down the range of possible responses displayed by a given host species to safe ones only. Effective parasite avoidance strategies could therefore be identified by carefully investigating the properties of animal species with unusually few parasites compared to related species with rich parasite faunas.

EVOLUTIONARY COVARIANCE OF AVOIDANCE TRAITS VERSUS INFECTION RISK

Could the fixation of parasite avoidance strategies, originally evolved out of "fear" and to counter immediate risks of infection, but later maintained because they serve other beneficial functions, obscure the relationship between host traits and parasite species richness? Many host traits, such as diet specialization, whether they are parasite avoidance strategies or coincidental determinants of parasite exposure, are expected to correlate with parasite species richness across host species. However, comparative analyses reveal no consistent correlations between diet breadth and parasite species richness across various groups of vertebrate hosts: the relationship is significant in some studies, but not others (e.g., Gregory et al., 1991; Watve and Sukumar, 1995; Sasal et al., 1997; Morand et al., 2000; Nunn et al., 2003). The same is true of many other host traits thought to be associated with parasite species richness (Kamiya et al., 2014; Morand, 2015).

We suggest that the relationship between parasite infection risk and the phenotypic expression of any given parasite avoidance trait may be reversed over evolutionary time (**Figure 2**). When a clade of hosts first encounters a new set of parasites, following a host shift or habitat invasion, host populations or host species facing the highest infection risk, i.e., the most parasite species, will evolve the strongest avoidance response. This should produce a positive relationship, across host populations or species, between infection risk and the degree to which avoidance strategies are expressed. However, this situation is dynamic over evolutionary time, as hosts and parasites are involved in a coevolutionary arms race. Just as hosts evolve parasite avoidance strategies, parasites will evolve counter-adaptations. Although, if the costs of overcoming the avoidance strategies of a host species are too high, a parasite may simply opt out of the race. Thus, if members of a host species become too rarely encountered or too difficult to infect, thanks to their avoidance strategies, selection may favor parasites that drop this host species from the range of hosts they can exploit, saving themselves the cost of species-specific adaptations against certain host defensive systems. Indeed, there are costs of being a generalist parasite, because maintaining adaptations to exploit multiple host species often limits performance on any of them: jacks of all trades are usually master of none (Straub et al., 2011; Arbiv et al., 2012; Pinheiro et al., 2016). Thus, short-term success at avoiding parasites could lead to a long-term reduction of the number of parasites actually exploiting a host species, and thus a negative relationship, across host populations or species, between infection risk and the expression of avoidance strategies (**Figure 2**). This may not only apply to traits playing a role pre-infection, such as avoidance strategies, but also to traits acting post-infection, such as those involved in immunocompetence like relative spleen sizes in birds (e.g., Morand and Poulin, 2000).

Of course, the above scenario is not the only possible evolutionary direction. If a particular avoidance trait is costly and traded-off against other functions, and if pressure from infection risk is relaxed, we might expect the expression of the trait to be reduced with time. In contrast, if it is co-opted for other functions and continues to confer benefits, it may persist and become disconnected from its original parasite avoidance function. Or, if it is fitness neutral, the trait may persist even with no clear function. Nevertheless, the scenario we propose could explain the lack of consistent relationships found between parasite species richness and host traits thought to be associated with infection risk: the various studies focusing on different taxa may simply capture snapshots of different stages in a dynamic evolutionary process.

THE CAUSALITY CHAIN

Any association among extant species between a particular trait and some measure of infection risk like parasite species richness can have at least two evolutionary explanations. Some host species may have few parasite species because that trait provides them with limited exposure, with the trait having evolved for other reasons or functions in the first place. Alternatively, the trait may have evolved to its full extent in those host species that faced a high infection risk and specifically for the purpose of lowering that risk, even if these hosts now experience a much lower infection risk. For instance, among Amazonian bats, omnivorous species have much higher infection levels by foodborne helminths than species that specialize strictly on nectar or fruits (Albuquerque et al., 2016). One possible explanation is that the narrow diet of a specialized forager could coincidentally protect it from exposure to many foodborne parasites, even if that diet has evolved for other ecological or physiological reasons. Alternatively, a forager living in an environment rich in foodborne parasites may have been selected for a narrow diet as a parasite avoidance strategy. How can we distinguish between these two scenarios?

Correlative evidence from field studies can only support the "ghost of parasitism past" hypothesis, but it cannot refute it. As an example, consider the link between fish migration and infection risk. In a field study of sympatric and congeneric species of galaxiid fishes in New Zealand, species that leave freshwater habitats and migrate downstream to spend their early life in coastal habitats incur significantly lower risks of infection by a range of trematode species, compared to their close relatives which do not migrate and reside in freshwaters for their whole lives (Poulin et al., 2012). There are clear benefits gained by fish that avoid trematode infection in early life: infection of young fish <40 mm in length causes malformations and greatly increases mortality (Kelly et al., 2010). So why do these fish migrate? Is the migratory behavior truly acting as an ongoing strategy to reduce exposure to parasites during a critical life stage? Or is it the legacy of an ancestral parasite avoidance strategy retained by selection for the other benefits it confers? Or did it evolve for completely different reasons and only coincidentally provides reduced infection risk as a side-effect? Theoretical models suggest that parasitism can indeed exert selective pressures strong enough to drive the evolution of migration, but only if it causes a reduction in prevalence by costly parasites rather than a reduction in the number of parasite species faced by the fish (Shaw et al., 2018, 2019).

Similar comparative studies among related species that differ in a key trait also hint at past selective pressures from parasites, with species possessing particular traits successfully escaping from parasitism. For instance, lepidopteran species with hairy caterpillars face a much lower risk of attack by parasitoid wasps than those with smooth caterpillars (Kageyama and Sugiura, 2016), as do species of gall wasps which produce ornate and bumpy galls compared to those that produce simpler galls (Bailey et al., 2009). The huge variation in behavioral and morphological defenses among insects (Gross, 1993) provides excellent material for tests of the "ghost of parasitism past" hypothesis.

Although intriguing, the results of comparative studies on extant species provide only hints of what may have happened over evolutionary time. Studies that focus on changes across long time scales would be more convincing, but these also have their limitations. In particular, we lack data on the strength of selective pressures exerted by parasites on extinct animals in the deep past. Data on infection risk or parasite species richness in extinct host species are almost impossible to obtain, except from glimpses obtained from coprolites (e.g., Wood et al., 2013) or traces left on fossils (e.g., Huntley et al., 2014). Therefore, we are blind to past selection pressures, just as we are blind to most parasite avoidance strategies of extinct animals involving behavior or other phenotypes that leave no fossil traces.

Some approaches nevertheless allow large-scale and convincing ways of testing the "ghost of parasitism past" hypothesis. One of them would be to test for covariation between the geographical distribution of a putative parasite avoidance trait and the distribution of past selective pressures from parasites, estimated as accurately as possible, among populations of the same or closely related species. For example, the sickle cell hemoglobin gene causes malformation of red blood cells and reduces their ability to bind and transport oxygen (Serjeant and Serjeant, 2001). The gene provides some resistance to malaria in heterozygous carriers, but causes sickle cell anemia and is usually fatal in individuals carrying two copies of the gene. Geographical variation in the frequency of the gene among human populations shows a strong association with the historical distribution of malaria endemicity, dating back to the times before any interventions or control measures were first implemented (Piel et al., 2010). Yet the gene still persists in human populations from areas where malaria is no longer a problem. This provides strong evidence that even where an otherwise deleterious anti-parasite trait is no longer needed nor beneficial in any other way, it can persist as a signature of past selective pressures from parasites. A similar approach could be used to determine whether the expression of any presumed parasite avoidance trait shows a spatial match with estimates of past selective pressures. Threespine sticklebacks, *Gasterosteus aculeatus*, may be the ideal system for such an approach. They have proven an excellent model system for studies of host-parasite interactions (Barber, 2013), the diversity and abundance of parasites vary widely among stickleback populations (Poulin et al., 2011a), and the genetic and phenotypic signature of selection pressures from parasites has proven tractable in stickleback populations (Brunner et al., 2017; Weber et al., 2017).

Alternatively, one might use a comparative approach across host species that vary in the expression of the presumed avoidance trait. Using dated phylogenies as a basis for the comparative analysis, it may be possible to reconstruct the ancestral origin and evolution of the presumed avoidance trait, and use this information to predict whether a positive or negative correlation would be expected between trait expression and relative parasite infection risk (see previous section). None of the approaches summarized above is ideal on its own, but in combination they offer a way forward to put the hypothesis to the test.

CONCLUSION

In this essay, we have argued that what begins as an adaptation providing immediate protection from infection may in the long run promote the loss of parasites and lead to a host species with fewer natural enemies. An inducible parasite avoidance mechanism showing variation among individuals could produce, if parasite pressures are sustained over many generations, fixed species-wide traits and/or an evolutionary niche shift. These can manifest in many ways. For instance, one could even argue that the excessive propensity for hygiene among people in industrialized countries is a legacy of pressures from parasites no longer plaguing human populations. In homage to previous hypotheses explaining niche segregation in the absence of measurable competition among sympatric species from the same guild (Connell, 1980), or anti-predator adaptations displayed by populations no longer facing predation (Peckarsky and Penton, 1988; Gliwicz and Jachner, 1992), we dub this the ghost of parasitism past.

Of course, some caveats apply to the arguments we propose. For instance, a strategy protecting hosts against a parasite that is no longer present may nevertheless persist if it also protects the host against other parasites. Alternatively, an avoidance strategy that serves against parasites that go extinct may be underpinned by the same neurobiological and physiological mechanisms that allow other avoidance strategies acting against other natural enemies that are still around; that strategy would therefore not necessarily be abandoned. The general avoidance of feces shown by many animals belongs to this category (Weinstein et al., 2018). The "ghost of parasitism past" hypothesis thus requires a more nuanced interpretation than what we presented in some of the earlier examples.

Due to the nature of the phenomenon, the "ghost of parasitism past" hypothesis cannot be tested within an experimental framework allowing strong inference of causality. However, we provide plausible examples of situations where putative avoidance strategies may now be species characteristics associated with fewer parasite species than one would expect based on a host species' other ecological features or phylogenetic affinities. We also propose some analytical approaches which, though not perfect, can serve to support the hypothesis. The morphology and behavior of extant animals display an array of adaptations that evolved in response to sustained pressures from predators, ranging from cryptic coloration and spines, to vigilance and alarm calls. Some of these persist in populations where predation pressures have been relaxed. We argue that similar adaptations against past pressures from parasites must be widespread in the animal kingdom. These are perhaps more subtle and more difficult to identify than adaptations against predators, but they are no less worthy of study.

AUTHOR CONTRIBUTIONS

All authors actively participated in developing the concepts presented in the manuscript. RP wrote the manuscript, with input from all other authors.

FUNDING

JB, DA, J-FD, AF, EP, and BR were funded by Doctoral Scholarships from the University of Otago. J-FD was also funded by a scholarship (PGSD3-530445-2019) from the Natural Sciences and Engineering Research Council of Canada (NSERC).

ACKNOWLEDGMENTS

We thank Janet Koprivnikar for inviting us (on behalf of the other editors) to contribute to this special issue. We also thank Tommy Leung and Chris Selbach for comments on an earlier version of the manuscript.

REFERENCES

Albuquerque, A. C. A., Moraes, M. F. D., Silva, A. C., Lapera, I. M., Tebaldi, J. H., and Hoppe, E. G. L. (2016). Helminth fauna of chiropterans in Amazonia: biological interactions between parasite and host. *Parasitol. Res.* 115, 3229–3237. doi: 10.1007/s00436-016-5085-3

Arbiv, A., Khokhlova, I. S., Ovadia, O., Novoplansky, A., and Krasnov, B. R. (2012). Use it or lose it: reproductive implications of ecological specialization in a haematophagous ectoparasite. *J. Evol. Biol.* 25, 1140–1148. doi: 10.1111/j.1420-9101.2012.02499.x

Bailey, R., Schönrogge, K., Cook, J. M., Melika, G., Csoka, G., Thuroczy, C., et al. (2009). Host niches and defensive extended phenotypes structure parasitoid wasp communities. *PLoS Biol.* 7:e1000179. doi: 10.1371/journal.pbio.1000179

Barber, I. (2013). Sticklebacks as model hosts in ecological and evolutionary parasitology. *Trends Parasitol.* 29, 556–566. doi: 10.1016/j.pt.2013.09.004

Barlow, C. C. (2000). *The Ghosts of Evolution*. New York, NY: Basic Books.

Bordes, F., and Morand, S. (2009). Parasite diversity: an overlooked metric of parasite pressures? *Oikos* 118, 801–806. doi: 10.1111/j.1600-0706.2008.17169.x

Brunner, F. S., Anaya-Rojas, J. M., Matthews, B., and Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. *Proc. Natl. Acad. Sci. U.S.A.* 114, 3678–3683. doi: 10.1073/pnas.1619147114

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

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

Connell, J. H. (1980). Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos* 35, 131–138.

Fritzsche, A., and Allan, B. F. (2012). The ecology of fear: Host foraging behavior varies with the spatio-temporal abundance of a dominant ectoparasite. *Ecohealth* 9, 70–74. doi: 10.1007/s10393-012-0744-z

Gliwicz, Z. M., and Jachner, A. (1992). Diel migrations of juvenile fish: a ghost of predation past or present. *Arch. Hydrobiol.* 124, 385–410.

Gregory, R. D., Keymer, A. E., and Harvey, P. H. (1991). Life history, ecology and parasite community structure in Soviet birds. *Biol. J. Linn. Soc.* 43, 249–262. doi: 10.1111/j.1095-8312.1991.tb00597.x

Gross, P. (1993). Insect behavioral and morphological defences against parasitoids. *Annu. Rev. Entomol.* 38, 251–273. doi: 10.1146/annurev.en.38.010193.001343

Hamilton, W. D. (1980). Sex versus non-sex versus parasite. *Oikos* 35, 282–290.

Hughes, J., and Page, R. D. M. (2007). Comparative tests of ectoparasite species richness in seabirds. *BMC Evol. Biol.* 7:227. doi: 10.1186/1471-2148-7-227

Huntley, J. W., Fürsich, F. T., Alberti, M., Hethke, M., and Liu, C. (2014). A complete Holocene record of trematode-bivalve infection and implications for the response of parasitism to climate change. *Proc. Nat. Acad. Sci. U.S.A.* 111, 18150–18155. doi: 10.1073/pnas.1416747111

Kageyama, A., and Sugiura, S. (2016). Caterpillar hairs as an anti-parasitoid defence. *Sci. Nat.* 103, 86.

Kamiya, T., O'Dwyer, K., Nakagawa, S., and Poulin, R. (2014). What determines species richness of parasitic organisms? A meta-analysis across animal, plant and fungal hosts. *Biol. Rev.* 89, 123–134. doi: 10.1111/brv.12046

Kavaliers, M., and Choleris, E. (2018). The role of social cognition in parasite and pathogen avoidance. *Philos. Trans. R. Soc. B* 373:20170206. doi: 10.1098/rstb.2017.0206

Kelly, D. W., Thomas, H., Thieltges, D. W., Poulin, R., and Tompkins, D. M. (2010). Trematode infection causes malformations and population effects in a declining New Zealand fish. *J. Anim. Ecol.* 79, 445–452. doi: 10.1111/j.1365-2656.2009.01636.x

Morand, S. (2015). (macro-)Evolutionary ecology of parasite diversity: from determinants of parasite species richness to host diversification. *Int. J. Parasitol. Parasit. Wildl.* 4, 80–87. doi: 10.1016/j.ijppaw.2015.01.001

Morand, S., Cribb, T. H., Kulbicki, M., Chauvet, C., Dufour, V., Faliex, E., et al. (2000). Determinants of endoparasite species richness of New Caledonian Chaetodontidae. *Parasitology* 121, 65–73. doi: 10.1017/s0031182099006058

Morand, S., and Poulin, R. (2000). Nematode parasite species richness and the evolution of spleen size in birds. *Can. J. Zool.* 78, 1356–1360. doi: 10.1139/z00-076

Norris, K. (1999). A trade-off between energy intake and exposure to parasites in oystercatchers feeding on a bivalve mollusc. *Proc. R. Soc. B* 266, 1703–1709. doi: 10.1098/rspb.1999.0835

Nunn, C. L., Altizer, S., Jones, K. E., and Sechrest, W. (2003). Comparative tests of parasite species richness in primates. *Am. Nat.* 162, 597–614. doi: 10.1086/378721

Peckarsky, B. L., and Penton, M. A. (1988). Why do *Ephemerella nymphs* scorpion posture: a "ghost of predation past"? *Oikos* 53, 185–193.

Piel, F. B., Patil, A. P., Howes, R. E., Nyangiri, O. A., Gething, P. W., Williams, T. N., et al. (2010). Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nat. Commun.* 1:104.

Pinheiro, R. B. P., Félix, G. M. F., Chaves, A. V., Lacorte, G. A., Santos, F. R., Braga, E. M., et al. (2016). Trade-offs and resource breadth processes as drivers of performance and specificity in a host-parasite system: a new integrative hypothesis. *Int. J Parasitol.* 46, 115–121. doi: 10.1016/j.ijpara.2015.10.002

Poulin, R., Blanar, C. A., Thieltges, D. W., and Marcogliese, D. J. (2011a). The biogeography of parasitism in sticklebacks: distance, habitat differences and the similarity in parasite occurrence and abundance. *Ecography* 34, 540–551. doi: 10.1111/j.1600-0587.2010.06826.x

Poulin, R., Guilhaumon, F., Randhawa, H. S., Luque, J. L., and Mouillot, D. (2011b). Identifying hotspots of parasite diversity from species-area relationships: host phylogeny versus host ecology. *Oikos* 120, 740–747. doi: 10.1111/j.1600-0706.2010.19036.x

Poulin, R., Closs, G. P., Lill, A. W. T., Hicks, A. S., Herrmann, K. K., and Kelly, D. W. (2012). Migration as an escape from parasitism in New Zealand galaxiid fishes. *Oecologia* 169, 955–963. doi: 10.1007/s00442-012-2251-x

Preisser, E. L., Bolnick, D. I., and Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* 87, 501–509. doi: 10.1890/04-0719

Randhawa, H. S., and Poulin, R. (2010). Determinants of tapeworm species richness in elasmobranch fishes: untangling environmental and phylogenetic influences. *Ecography* 33, 866–877. doi: 10.1111/j.1600-0587.2010.06169.x

Sasal, P., Morand, S., and Guégan, J. F. (1997). Determinants of parasite species richness in Mediterranean marine fish. *Mar. Ecol. Progr. Ser.* 149, 61–71. doi: 10.3354/meps149061

Serjeant, G. R., and Serjeant, B. E. (2001). *Sickle Cell Disease*. Oxford: Oxford University Press.

Shaw, A. K., Craft, M. E., Zuk, M., and Binning, S. A. (2019). Host migration strategy is shaped by forms of parasite transmission and infection cost. *J. Anim. Ecol.* 88, 1601–1612. doi: 10.1111/1365-2656.13050

Shaw, A. K., Sherman, J., Barker, F. K., and Zuk, M. (2018). Metrics matter: the effect of parasite richness, intensity and prevalence on the evolution of host migration. *Proc. R. Soc. B* 285:20182147. doi: 10.1098/rspb.2018.2147

Straub, C. S., Ives, A. R., and Gratton, C. (2011). Evidence for a trade-off between host-range breadth and host-use efficiency in aphid parasitoids. *Am. Nat.* 177, 389–395. doi: 10.1086/658177

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.

Watve, M. G., and Sukumar, R. (1995). Parasite abundance and diversity in mammals: correlates with host ecology. *Proc. Nat. Acad. Sci. U.S.A.* 92, 8945–8949. doi: 10.1073/pnas.92.19.8945

Weber, J. N., Kalbe, M., Shim, K. C., Erin, N. I., Steinel, N. C., Ma, L., et al. (2017). Resist globally, infect locally: a transcontinental test of adaptation by stickleback and their tapeworm parasite. *Am. Nat.* 189, 43–57. doi: 10.1086/689597

Weinstein, S. B., Buck, J. C., and Young, H. S. (2018). A landscape of disgust. *Science* 359, 1213–1214. doi: 10.1126/science.aas8694

Wilson, K., Fenton, A., and Tompkins, D. (2019). *Wildlife Disease Ecology*. Cambridge: Cambridge University Press.

Wood, J. R., Wilmshurst, J. M., Rawlence, N. J., Bonner, K. I., Worthy, T. H., Kinsella, J. M., et al. (2013). A megafauna's microfauna: gastrointestinal parasites of New Zealand's extinct moa (Aves: Dinornithiformes). *PLoS One* 8:e57315. doi: 10.1371/journal.pone.0057315

Zanette, L. Y., and Clinchy, M. (2019). Ecology of fear. *Curr. Biol.* 29, R309–R313.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

PERSPECTIVE
published: 18 September 2020
doi: 10.3389/fevo.2020.564343

An Integrated Landscape of Fear and Disgust: The Evolution of Avoidance Behaviors Amidst a Myriad of Natural Enemies

Jean-François Doherty* and Brandon Ruehle

Department of Zoology, University of Otago, Dunedin, New Zealand

Fear of natural enemies in non-human animals is a concept dating back to the time of Darwin. Now recognized as a non-consumptive effect, the ecological and evolutionary impact of fear has been studied in a number of predator-prey systems within the last few decades. However, comparatively little consideration has been given to the non-consumptive effects that parasites have on their hosts, which have evolved behaviors to avoid parasites, impacting habitat selection, mate choice, and foraging activity. These avoidance behaviors create a "landscape of disgust," wherein hosts navigate to avoid parasites, akin to the "landscape of fear" in prey. Evolutionarily speaking, however, predators and parasites are but two examples of natural enemies. Pathogens, parasites, predators, and parasitoids, among others, each exert their own fitness cost on the victims they attack. Since animals likely evolve in the presence of multiple natural enemies, they must adopt a range of avoidance behaviors to navigate through the resulting "landscape of peril." Therefore, in line with recent efforts to combine the landscapes of fear and disgust, we offer a theoretical framework to better understand the impacts of natural enemies on the evolution of trait-mediated avoidance behaviors in animals. More precisely, we look at how victims may evolve to allocate energy optimally among distinct avoidance behaviors under the selective pressures imposed by different types of natural enemy. This framework is then put into the more realistic context of a food web, which highlights the impact of trophic interactions and trophic level on the evolution of avoidance behaviors.

Keywords: avoidance behavior, fear, disgust, natural enemy, predator, parasite, non-consumptive effect

OPEN ACCESS

Edited by:
Janet Koprivnikar,
Ryerson University, Canada

Reviewed by:
Hillary Young,
University of California,
Santa Barbara, United States
Meredith Palmer,
Princeton University, United States

****Correspondence:***
Jean-François Doherty
jeff-doherty@hotmail.com

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *21 May 2020*
Accepted: *02 September 2020*
Published: *18 September 2020*

Citation:
Doherty J-F and Ruehle B (2020)
An Integrated Landscape of Fear
and Disgust: The Evolution
of Avoidance Behaviors Amidst
a Myriad of Natural Enemies.
Front. Ecol. Evol. 8:564343.
doi: 10.3389/fevo.2020.564343

INTRODUCTION

When imagining a pride of lions chasing down an antelope in sub-Saharan Africa, few would consider that these apex predators have any enemies of their own. As it turns out, even the "king of the jungle" has its own set of natural enemies [i.e., any organism that has evolved to exploit the resources of a victim, incurring a negative or lethal impact on the latter (Raffel et al., 2008)] which, apart from humans and sometimes hyenas, are mostly parasites (Bjork et al., 2000; Berentsen et al., 2012). In fact, it is likely that every metazoan species has evolved with at least one parasite (Poulin, 2014; Costello, 2016), therefore it is reasonable to assume that no animal species is devoid of natural enemies (now referred to simply as enemies). Following this assumption, we would expect lions to have evolved traits that reduce the probability of acquiring parasites, such as avoiding the carcasses of conspecifics and other carnivores even though they represent an easy energy source (Moléon et al., 2017). Avoidance behaviors such as this are termed non-consumptive effects because the victim (i.e., the lion) is responding directly to a potential act of consumption by an enemy (i.e., a parasite), without consumption actually taking place (Curtis, 2014; Buck et al., 2018). Further, if lions evolved behaviors to mostly avoid parasites, antelopes may have evolved an even greater number of behaviors to avoid lions, other predators, and parasites.

For a primary consumer like the antelope, the non-consumptive effects of predation on their reproduction and even survival can sometimes surpass the direct effects of consumption (Sheriff and Thaler, 2014; MacLeod et al., 2018). These processes are now well established in predator-prey interactions (Gaynor et al., 2019), even though the overall impacts on prey population size are still poorly understood (Sheriff et al., 2020). Parasites can also induce non-consumptive effects in their hosts, a topic that has received more recognition in recent years (Sarabian et al., 2018b; Buck, 2019). Hosts can avoid getting infected through changes in behavior (Hart and Hart, 2018), such as limiting interactions with conspecifics that appear infected (Stephenson et al., 2018), directly avoiding parasitic infective stages through the detection of cues (Strauss et al., 2019), or changing feeding habits (Sarabian et al., 2018a) or habitat use (Amoroso et al., 2019). These avoidance behaviors differ from other defensive strategies in that they are a direct and dynamic response to environmental cues, as opposed to constitutional defense strategies such as morphology (e.g., the long hairs on a caterpillar). As a result, hosts have evolved to invest resources in behavioral or sensory traits that decrease the probability of infection. Animals can thus navigate more safely through their environment, which has aptly been named the "landscape of disgust" (Weinstein et al., 2018a) as opposed to the "landscape of fear" in predator-prey systems (Laundré et al., 2001). The degree to which a species experiences fear or disgust is difficult to assess (Mendl et al., 2010), but regardless of the avoidance behavior, it is likely that many species have evolved with more than one enemy to contend with.

As enemies go, predators and parasites are but broad generalizations of a multitude of trophic strategies that exist, each one having its own fitness cost on the victims that are successfully attacked (Lafferty and Kuris, 2002). For example, parasitic castrators do not kill their hosts, but they effectively suppress host reproduction, thereby reducing host fitness to zero (Lafferty and Kuris, 2009). Other enemies, such as micropredators (e.g., mosquitoes and vampire bats), may feed on multiple hosts without drastically reducing their fitness (Poulin, 2011). Animals that have evolved with multiple enemies, each with specific trophic strategies, may allocate their resources toward avoidance behaviors that balance immediate danger with potential fitness loss. Tadpoles, for example, prefer to forage in the presence of trophically transmitted parasites rather than predators, likely because infection is relatively less costly to fitness than being eaten (Koprivnikar and Penalva, 2015). Considering the above, it is sensible to combine the landscapes of fear and disgust into a broader landscape of enemies (Buck et al., 2018; Sarabian et al., 2018b), which we call here the "landscape of peril." Only then could we understand how animals invest energy in their evolutionary toolkit of avoidance behaviors to safely navigate under the near constant threat of attack.

In light of recent efforts to unite the landscapes of fear and disgust (Buck et al., 2018; Weinstein et al., 2018a), we discuss here the broad evolutionary implications of animals that evolve in the presence of one or more enemies with different types of trophic strategy, each of which incurring a differential fitness cost upon the victim. By illustrating how victims may evolve to detect enemy cues and invest their resources into avoidance behaviors to navigate safely through the landscape of peril, we can predict how animals allocate energy for optimal survival. This depends ultimately on the selective pressures or risk imposed by enemies, which can be measured with abundance, lethality, or species richness (Bordes and Morand, 2009). These predictions are then verified with recent examples in the literature, putting into evolutionary context the landscape of peril. We then explore the idea of how, like for lions and antelopes, the trophic level in a food web may impact the avoidance behaviors in animals, along with some examples from the literature. We show that, regardless of trophic level, it is likely that all animal species have inevitably evolved with their own set of enemies. However, the composition of enemy types for a particular victim could depend on its relative position in the food web, in addition to other characteristics such as body size or sociality. Thus, we provide a theoretical backdrop for the evolution of avoidance behaviors in animals subjected to the selective pressures imposed by one or more types of enemy.

A MYRIAD OF NATURAL ENEMIES

Trophic strategies in animals are not limited to predators and parasites. Lafferty and Kuris (2002) recognized a total of ten trophic strategists, consisting of seven types of parasites and three types of predators. Each strategist incurs a negative fitness cost on the victims that they effectively consume or attack (see Figure 1 in Lafferty and Kuris, 2002), which varies in relation to the evolutionary history between both antagonistic species. An enemy that successfully attacks a victim will either eliminate its fitness completely or reduce it partially. So, if victim fitness has a maximum value of 1 in a continuum ranging from 0 to 1 inclusively, an enemy, depending on its trophic strategy, can either reduce it to 0 (i.e., a "lethal" enemy) or reduce it to a number between 0 and 1 (i.e., a non-lethal enemy) (**Figure 1A**). Of course, there are exceptions to this (e.g., a host with an extraordinary number of non-lethal parasites may die), but on an evolutionary timescale, we argue that this dichotomy represents a strong selective pressure exerted by enemies on the evolution of avoidance behaviors in victims.

Upon consumption, enemies can reduce victim fitness either partially or completely, but this is not the only selective pressure imposed on victims. We hypothesize that another important evolutionary pressure is the probability of a successful attack on a victim, which depends on a number of variables, such as

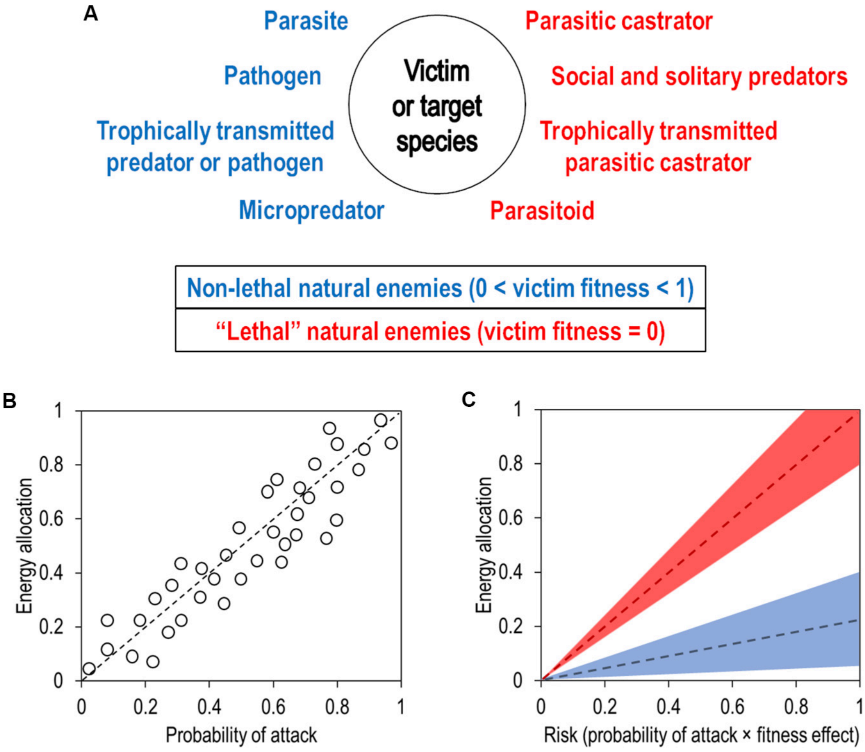

FIGURE 1 | How animal victims may evolve to allocate energy toward avoidance behaviors against natural enemies. **(A)** Ten recognized trophic strategies in natural enemies toward victims. "Lethal" natural enemies do not necessarily kill their victims, but effectively eliminate victim fitness. The dichotomy between lethal and non-lethal natural enemies represents one of the evolutionary pressures to evolve avoidance behaviors. **(B)** Theoretical evolution of energy allocation toward avoidance behaviors based solely on the probability that a victim will be attacked by a natural enemy during its lifetime. Each circle represents one victim population that evolves with a certain probability of being attacked by a natural enemy. **(C)** How victim populations theoretically evolve to allocate energy toward avoidance behaviors under the risk imposed by natural enemies. Risk results from the product of the probability of attack multiplied by the fitness cost imposed by a certain natural enemy. Therefore, in the case of victims that evolve exclusively with lethal natural enemies (red dashed line), the risk imposed by the latter equals to the probability of being attacked. With victims that evolve exclusively with non-lethal natural enemies (blue dashed line), the risk can range from anywhere between 0 and 1, depending on the fitness cost imposed by the natural enemy (in this case, the fitness cost is 0.2). The colored areas represent a plastic response of resource allocation, whereas the dashed lines represent a fixed response.

the population densities of both enemies and victims (Ioannou et al., 2008) and the resulting encounter rate between them (Mols et al., 2004). However, regardless of the determining factors, a victim is most likely subjected to a certain probability of being attacked by an enemy during its lifetime, ranging from 0 to 1 inclusively. In some host-parasite systems, the lifetime probability of being attacked is almost guaranteed to equal 1 (e.g., an abundant parasite present in the only water source of a particular area), therefore what matters in these cases is the probability of sufficient repeated attacks which results in an intensity of infection that lowers fitness. Nevertheless, if the probability of attack by an enemy remains constant or varies little over many generations, we predict that a victim will evolve to allocate resources toward their avoidance behaviors proportionally to this lifetime probability of attack (**Figure 1B**). This relationship may not be linear, but we would still expect a positive correlation between energy allocation and the probability of attack. If the probability of attack is more variable over evolutionary time, we would expect a more plastic response of resource allocation toward avoidance behaviors.

We suggest that victims have a risk of completely losing their fitness or dying from a lethal enemy that is equal to the lifetime probability of being successfully attacked by said enemy (**Figure 1C**). Therefore, a victim that evolves with a probability of 0.4 of being attacked by a lethal enemy during its lifetime should invest relatively less in their avoidance behaviors than a victim that evolves with a probability of 0.8 of being attacked (**Figure 1C**). Realistically, we could predict that this resource allocation translates into the amount of time that victims spend scanning their environment for enemy cues or how much they limit habitat use (see introduction). Regardless of the behavior employed, the more a victim invests resources into avoiding a potential enemy, the more likely it is to lose foraging opportunities. For example, deer that evolve with predators limit their foraging activities to twilight and night hours, whereas deer that have lived on predator-free islands for several generations forage more during the day, thus increasing their energy intake considerably (Bonnot et al., 2016). This appears to be due to a relaxed selection on foraging traits linked to predator avoidance. Still, the increased foraging activity during twilight hours is observed in deer on predator-free islands, suggesting that this avoidance trait persists even after long-term absence of predators. We could also predict that the energy investment into avoidance behaviors translates into the physiological sensitivity that victims have toward enemy cues, if the enemy is at all detectable. For example, pea aphids appear to respond rapidly to the presence of generalist parasitoids by dropping from the plant they are feeding on (Fill et al., 2012). Even though this species is not a host of the parasitoid, the aphid appears to be very sensitive to the presence of potential enemies. This high sensitivity results in the aphid losing a considerable amount of time away from the leaf, resulting in a reduction of energy intake.

The allocation of resources toward avoidance behaviors may differ if a victim evolves exclusively in the presence of a non-lethal enemy. Since they do not eliminate victim fitness upon consumption, the actual risk of dying (or potential loss of fitness) from a non-lethal enemy results from the product of the probability of attack multiplied by the fitness cost (**Figure 1C**). If non-lethal enemies impose the same amount of risk over time, victims should evolve to allocate resources accordingly. Therefore, even if non-lethal enemies pose less risk than lethal ones, we would still expect victims to invest resources into avoidance behaviors proportionally to the risk imposed by non-lethal enemies (e.g., lions avoiding carcasses, see introduction). As for lethal enemies, this relationship is certainly positive, but it may not be linear. As stated above, if the level of risk varies more over time, victims may evolve plastic traits to allocate energy responsively toward avoidance behaviors depending on perceived risk (**Figure 1C**). For example, the metabolic rate in *Drosophila* flies increases when they are either indirectly or directly exposed to parasitic mites, but to different degrees, suggesting that the avoidance behaviors of the fly may have evolved to contend with different levels of risk imposed by the enemy (Luong et al., 2017).

If a victim evolves with lethal and non-lethal enemies that impose similar levels of risk, we predict that more energy would be invested into avoiding the lethal enemy, simply because of the potential fitness loss it imposes. Even though both types of enemy can have a synergistic negative effect on victim survival (Marino and Werner, 2013; Shang et al., 2019), when given the choice, victims (e.g., tadpoles, see introduction) may choose to forage more under the threat of a non-lethal enemy than under the threat of a lethal one. This obviously depends on the risk associated with a certain enemy, which may vary in time and space. Moreover, victims that evolve plastic trait-mediated avoidance behaviors should be better at responding to the dynamic threats imposed by multiple types of enemy. Interestingly, some avoidance behaviors may evolve as a response to multiple enemy cues. A good example of this is the general avoidance of feces by animals that are susceptible to disease (Weinstein et al., 2018a). Victims under selective pressures to avoid both lethal and non-lethal enemies may have evolved traits to avoid feces altogether, which simultaneously reduces the risk of encountering predators and acquiring parasites (Weinstein et al., 2018b). However, the research on avoidance behaviors in response to both lethal and non-lethal enemies is limited to a few studies and even less study systems (Buck et al., 2018). Therefore, more research is needed to better understand the impacts of risk and enemy type on the evolution of avoidance behaviors in victims.

In this section, we argue that the dichotomy of fitness effects between lethal and non-lethal enemies and the lifelong probability of a successful attack represent the two strongest selective pressures imposed by enemies on the evolution of resource allocation toward avoidance behaviors. Of course, the probability of attack is variable and is also likely to be context-dependent, but if it remains somewhat constant over an evolutionary timescale, we would expect victims to evolve avoidance behaviors accordingly (see above). Another possible selective pressure imposed by enemies is the loss of energy intake or foraging opportunities due to the time victims spend detecting enemy cues or avoiding patches of food. While this indirect effect may also have implications in the evolution of avoidance behaviors, we suggest that a loss in energy intake results from the dynamic changes in behavior that evolved in response to the two pressures described above. Therefore, we argue that a loss of foraging opportunities is highly variable and context-dependent and represents a weaker selective pressure. For example, non-parasitized sheep avoid patches of vegetation contaminated with feces, which decreases food intake and activity levels. Contrarily, parasitized sheep graze more in contaminated patches, which increases food intake (Hutchings et al., 2001). Here, the trade-off between nutrition and parasitism appears to depend mainly on the infection status of the sheep.

WHAT ABOUT TROPHICALLY TRANSMITTED PARASITES?

Trophically transmitted parasites impose a unique type of selective pressure on victims. Their general life cycle includes at least one intermediate host that must then be consumed by a definitive host in order for the parasite to mature and reproduce (Poulin, 2011). These parasites can increase the likelihood that the intermediate host gets eaten by the definitive host through alteration of its behavior or appearance, making it more conspicuous (see Moore, 2002 for examples). For the intermediate host, this non-lethal enemy increases the probability of attack from a lethal enemy, meaning that the level of risk increases after infection. This evolutionary pressure may select for victims that allocate more resources toward avoiding trophically transmitted parasites, which also reduces the probability of being eaten by the definitive host of the parasite. For the definitive host, a conspicuous prey is easier to identify, providing more resources, however, this increase in energy intake also increases exposure to infection. Although trophically transmitted parasites are unlikely to have evolved to harm or kill their definitive hosts, since they help disperse the parasite into the environment, the host may have evolved traits to avoid heavily parasitized prey in order to mitigate the reward of eating conspicuous prey with the cost of acquiring heavy parasite burdens. For example, oystercatchers consume mainly medium-sized cockles and avoid larger, energy-rich cockles that are likelier to have heavy parasite loads (Norris, 1999).

UP AND DOWN THE FOOD WEB

Animals at the base or at the top of the food web probably evolve with different ratios of lethal to non-lethal enemies. If an apex predator, such as a lion, evolves avoidance behaviors mainly against non-lethal enemies like parasites (i.e., a lower relative number of lethal enemies), it is reasonable to suggest that, as we move down the food web, primary and secondary consumers may evolve avoidance behaviors mainly against lethal enemies (i.e., a higher relative number of lethal enemies). To test this idea, we look at food web data for a shallow brackish water ecosystem from Germany and Denmark (Zander et al., 2011). By counting the number of trophic links between victim species and enemy species provided in this study, it was possible to estimate the species richness of lethal and non-lethal enemies for each victim. Victim species were then grouped qualitatively into “organismal groups” identified by the authors of the study, in order to approximate the trophic level of each victim species. From these data, we are able to observe that the total number of lethal enemies, along with the ratio of lethal to non-lethal enemies, decrease in victims as we move up the food web of aquatic and semi-aquatic animal groups (**Figure 2** and **Supplementary Material**). This suggests that lower-level consumers, such as invertebrates and small vertebrates, probably evolve to invest more resources into avoidance behaviors against both non-lethal and lethal enemies than do tertiary consumers. Therefore, a primary consumer like an amphipod may invest heavily into avoiding lethal enemies, whereas a secondary consumer like a solitary fish may invest relatively more resources toward non-lethal ones. In fact, in this aquatic food web, the ratio of lethal to non-lethal enemies is almost completely inversed between primary consumers and tertiary ones (**Figure 2**). Here, the framework of the landscape of peril appears to apply more to victims situated in the middle trophic levels, which have to contend with both lethal and non-lethal enemies. Victims at the bottom of the food chain may navigate more through a landscape of fear, whereas victims at the top of the food chain may navigate more through a landscape of disgust.

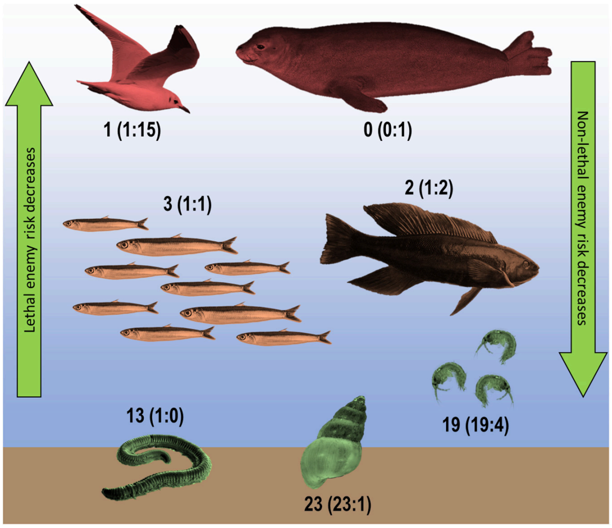

FIGURE 2 | Number of lethal natural enemy species (i.e., enemies that effectively eliminate the fitness of victims, such as predators and parasitic castrators) with the ratio of lethal to non-lethal natural enemy species (i.e., enemies that reduce fitness to between 0 and 1, such as trophically transmitted parasites and pathogens) in parentheses for different organismal animal groups (body size is not to scale) in a simplified food web of a tidal basin that approximates trophic level. From left to right, primary consumers in green include annelids, snails, and amphipods; secondary consumers in orange include schooling fishes and solitary fishes; tertiary consumers in red include birds and mammals. The numbers for each animal group are median values calculated for a number of species that were included in each group (**Supplementary Material**). Note that, for mammals, only one species was available from the dataset.

The complex interactions between species in a food web may change depending on how victims evolve to invest in their avoidance behaviors. For example, if a victim avoids conspecifics to reduce the likelihood of acquiring non-lethal enemies, this could simultaneously increase the likelihood of being detected and eaten by a lethal one. Rainbow trout tend to form smaller and less cohesive shoals in the presence of conspecifics infected with eye flukes (a trophically transmitted parasite), which may in turn increase the chance of individual trout getting eaten by birds (Seppälä et al., 2008). Here, there is a clear trade-off between parasitism and predation in the trout, with social grouping behaviors playing a key balancing role. Thus, the evolution of avoidance behaviors toward non-lethal enemies may drastically change the energy flow in trophic networks. It is recognized that parasites alone are important in sustaining diverse and complex food webs (Dunne et al., 2013). However, there remains much to be discovered about the hidden diversity of non-lethal enemies such as parasites and pathogens and the roles they play in maintaining the structure and health of food webs (Lafferty et al., 2008; Sukhdeo, 2012).

If a certain victim evolves with a particular set of enemies due mainly to its position in the food web, it is possible that others lessen this particular pressure by growing larger. For instance, mammal species that grow beyond a certain body mass threshold are less susceptible to predation, but may still evolve avoidance behaviors toward parasites (Sinclair et al., 2003). Asian elephants, primary consumers that are seldom predated upon (except perhaps for small juveniles), have evolved the avoidance behavior of “fly switching,” in which an individual fashions a swatting tool out of a tree branch to decrease the risk of getting bitten or infected by parasitic flies (Hart and Hart, 1994; Hart et al., 2001). Therefore, even if growing big reduces the probability of being attacked by a lethal enemy, larger victims may still invest energy in avoidance behaviors against smaller, non-lethal ones. Actually, the diversity of non-lethal enemies tends to increase for larger animal victim species. In a meta-analysis from Kamiya et al. (2014), it was shown that parasite species richness increases generally with host body size, a trend that appears to be universal across taxa and study scales. So, if larger animals harbor more diverse parasite communities than do smaller ones in general, it is reasonable to assume that they evolved to invest relatively more in their avoidance behaviors against non-lethal enemies.

CONCLUSION

All species likely evolve under the selective pressures imposed by enemies and therefore have to adapt in order to safely navigate through their environment with the use of avoidance behaviors. We argue that the lifelong probability of a successful attack and the fitness cost imposed by enemies (lethal or non-lethal) create risk, which ultimately selects for victims that allocate energy toward avoidance behaviors that reduce the risk of an enemy attack, thus optimizing survival. Both the fitness cost and the probability of attack represent strong selective pressures for the evolution of resource allocation toward avoidance behaviors. Moreover, depending on how variable the probability of attack is over evolutionary time, victims may adapt plastic trait-mediated behaviors to evade the dynamic pressures exerted by one or more enemies. We also argue that, depending on the position of a victim in the food web, resource allocation may be focused more into behaviors for avoiding lethal enemies as opposed to non-lethal ones, or vice versa.

In practice, we predict that the allocation of resources toward avoidance behaviors may translate into the amount of time that victims spend scanning their environment for enemy cues. Victims may also evolve to invest more or less of their time or energy avoiding certain areas or activities, based on perceived risk in the landscape of peril. Such activities could result in the loss of foraging opportunities and potential energy intake. Additionally, we predict that the evolution of resource allocation impacts the sensitivity of victims toward enemy cues. Therefore, depending on the pressures imposed by the enemies they evolve with, victims may be more or less attuned to the environmental signals left by enemies. To date, there are only a few studies that simultaneously look at the complex interactions between victims and their multiple types of enemies; the ones that do exist focus on a limited set of study systems (Buck et al., 2018). In sum, this evolutionary framework stresses the importance of considering the impacts of multiple enemies with differential fitness costs and levels of risk. There is still much to understand in this burgeoning field of research and this perspective will hopefully provide ideas for stimulating new studies about the landscape of peril.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

J-FD and BR conceptualized the idea of the manuscript. J-FD wrote the manuscript. BR critically revised it. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the University of Otago Doctoral Scholarships to the authors and a Natural Sciences and Engineering Research Council of Canada (NSERC) scholarship (PGSD3-530445-2019) to J-FD.

ACKNOWLEDGMENTS

We thank JK for the invitation (on behalf of the other topic editors) to contribute to this Research Topic. We also thank Robert Poulin for feedback and constructive criticism on an earlier draft.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.564343/full#supplementary-material

REFERENCES

Amoroso, C. R., Kappeler, P. M., Fichtel, C., and Nunn, C. L. (2019). Fecal contamination, parasite risk, and waterhole use by wild animals in a dry deciduous forest. *Behav. Ecol. Sociobiol.* 73:153. doi: 10.1007/s00265-019-2769-6

Berentsen, A. R., Becker, M. S., Stockdale-Walden, H., Matandiko, W., McRobb, R., and Dunbar, M. R. (2012). Survey of gastrointestinal parasite infection in African lion (*Panthera leo*), African wild dog (*Lycaon pictus*) and spotted hyaena (*Crocuta crocuta*) in the Luangwa Valley, Zambia. *Afr. Zool.* 47, 363–368. doi: 10.3377/004.047.0204

Bjork, K. E., Averbeck, G. A., and Stromberg, B. E. (2000). Parasites and parasite stages of free-ranging wild lions (*Panthera leo*) of northern Tanzania. *J. Zoo Wildl. Med.* 31, 56–61. doi: 10.1638/1042-7260(2000)031[0056:papsof]2.0.co;2

Bonnot, N. C., Morellet, N., Hewison, A. J. M., Martin, J.-L., Benhamou, S., and Chamaille-Jammes, S. (2016). Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) adjust habitat selection and activity rhythm to the absence of predators. *Can. J. Zool.* 94, 385–394. doi: 10.1139/cjz-2015-0227

Bordes, F., and Morand, S. (2009). Parasite diversity: an overlooked metric of parasite pressures? *Oikos* 118, 801–806. doi: 10.1111/j.1600-0706.2008.17169.x

Buck, J. C. (2019). Indirect effects explain the role of parasites in ecosystems. *Trends Parasitol.* 35, 835–847. doi: 10.1016/j.pt.2019.07.007

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

Costello, M. J. (2016). Parasite rates of discovery, global species richness and host specificity. *Integr. Comp. Biol.* 56, 588–599. doi: 10.1093/icb/icw084

Curtis, V. A. (2014). Infection-avoidance behaviour in humans and other animals. *Trends Immunol.* 35, 457–464. doi: 10.1016/j.it.2014.08.006

Dunne, J. A., Lafferty, K. D., Dobson, A. P., Hechinger, R. F., Kuris, A. M., Martinez, N. D., et al. (2013). Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biol.* 11:e1001579. doi: 10.1371/journal.pbio.1001579

Fill, A., Long, E. Y., and Finke, D. L. (2012). Non-consumptive effects of a natural enemy on a non-prey herbivore population. *Ecol. Entomol.* 37, 43–50. doi: 10.1111/j.1365-2311.2011.01333.x

Gaynor, K. M., Brown, J. S., Middleton, A. D., Power, M. E., and Brashares, J. S. (2019). Landscapes of fear: spatial patterns of risk perception and response. *Trends Ecol. Evol.* 34, 355–368. doi: 10.1016/j.tree.2019.01.004

Hart, B. L., and Hart, L. A. (1994). Fly switching by Asian elephants: tool use to control parasites. *Anim. Behav.* 48, 35–45. doi: 10.1006/anbe.1994.1209

Hart, B. L., and Hart, L. A. (2018). How mammals stay healthy in nature: the evolution of behaviours to avoid parasites and pathogens. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170205. doi: 10.1098/rstb.2017.0205

Hart, B. L., Hart, L. A., McCoy, M., and Sarath, C. R. (2001). Cognitive behaviour in Asian elephants: use and modification of branches for fly switching. *Anim. Behav.* 62, 839–847. doi: 10.1006/anbe.2001.1815

Hutchings, M. R., Gordon, I. J., Kyriazakis, I., and Jackson, F. (2001). Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake rate of forage and parasitism in subsequent foraging decisions. *Anim. Behav.* 62, 955–964. doi: 10.1006/anbe.2001.1837

Ioannou, C. C., Ruxton, G. D., and Krause, J. (2008). Search rate, attack probability, and the relationship between prey density and prey encounter rate. *Behav. Ecol.* 19, 842–846. doi: 10.1093/beheco/arn038

Kamiya, T., O'Dwyer, K., Nakagawa, S., and Poulin, R. (2014). What determines species richness of parasitic organisms? A meta-analysis across animal, plant and fungal hosts. *Biol. Rev.* 89, 123–134. doi: 10.1111/brv.12046

Koprivnikar, J., and Penalva, L. (2015). Lesser of two evils? Foraging choices in response to threats of predation and parasitism. *PLoS One* 10:e0116569. doi: 10.1371/journal.pone.0116569

Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., de Leo, G., Dobson, A. P., et al. (2008). Parasites in food webs: the ultimate missing links. *Ecol. Lett.* 11, 533–546. doi: 10.1111/j.1461-0248.2008.01174.x

Lafferty, K. D., and Kuris, A. M. (2002). Trophic strategies, animal diversity and body size. *Trends Ecol. Evol.* 17, 507–513. doi: 10.1016/s0169-5347(02)02615-0

Lafferty, K. D., and Kuris, A. M. (2009). Parasitic castration: the evolution and ecology of body snatchers. *Trends Parasitol.* 25, 564–572. doi: 10.1016/j.pt.2009.09.003

Laundré, J. W., Hernández, L., and Altendorf, K. B. (2001). Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, USA. *Can. J. Zool.* 79, 1401–1409. doi: 10.1139/cjz-79-8-1401

Luong, L. T., Horn, C. J., and Brophy, T. (2017). Mitey costly: energetic costs of parasite avoidance and infection. *Physiol. Biochem. Zool.* 90, 471–477. doi: 10.1086/691704

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

Marino, J. A., and Werner, E. E. (2013). Synergistic effects of predators and trematode parasites on larval green frog (*Rana clamitans*) survival. *Ecology* 94, 2697–2708. doi: 10.1890/13-0396.1

Mendl, M., Burman, O. H. P., and Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. *Proc. R. Soc. B Biol. Sci.* 277, 2895–2904. doi: 10.1098/rspb.2010.0303

Moléon, M., Martinez-Carrasco, C., Muellerklein, O. C., Getz, W. M., Muñoz-Lozano, C., and Sánchez-Zapata, J. A. (2017). Carnivore carcasses are avoided by carnivores. *J. Anim. Ecol.* 86, 1179–1191. doi: 10.1111/1365-2656.12714

Mols, C. M. M., van Oers, K., Witjes, L. M. A., Lessells, C. M., Drent, P. J., and Visser, M. E. (2004). Central assumptions of predator-prey models fail in a semi-natural experimental system. *Proc. R. Soc. B Biol. Sci.* 271, S85–S87. doi: 10.1098/rsbl.2003.0110

Moore, J. (2002). *Parasites and the Behavior of Animals*. Oxford: Oxford University Press.

Norris, K. (1999). A trade-off between energy intake and exposure to parasites in oystercatchers feeding on a bivalve mollusc. *Proc. R. Soc. B Biol. Sci.* 266, 1703–1709. doi: 10.1098/rspb.1999.0835

Poulin, R. (2011). The many roads to parasitism: a tale of convergence. *Adv. Parasitol.* 74, 1–40. doi: 10.1016/B978-0-12-385897-9.00001-X

Poulin, R. (2014). Parasite biodiversity revisited: frontiers and constraints. *Int. J. Parasitol.* 44, 581–589. doi: 10.1016/j.ijpara.2014.02.003

Raffel, T. R., Martin, L. B., and Rohr, J. R. (2008). Parasites as predators: unifying natural enemy ecology. *Trends Ecol. Evol.* 23, 610–618. doi: 10.1016/j.tree.2008.06.015

Sarabian, C., Belais, R., and Maclntosh, A. J. J. (2018a). Feeding decisions under contamination risk in bonobos. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170195. doi: 10.1098/rstb.2017.0195

Sarabian, C., Curtis, V., and McMullan, R. (2018b). Evolution of pathogen and parasite avoidance behaviours. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170256. doi: 10.1098/rstb.2017.0256

Seppälä, O., Karvonen, A., and Valtonen, E. T. (2008). Shoaling behaviour of fish under parasitism and predation risk. *Anim. Behav.* 75, 145–150. doi: 10.1016/j.anbehav.2007.04.022

Shang, G.-Z., Zhu, Y.-H., Wu, Y., Cao, Y.-F., and Bian, J.-H. (2019). Synergistic effects of predation and parasites on the overwinter survival of root voles. *Oecologia* 191, 83–96. doi: 10.1007/s00442-019-04455-4

Sheriff, M. J., Peacor, S. D., Hawlena, D., and Thaker, M. (2020). Non-consumptive predator effects on prey population size: a dearth of evidence. *J. Anim. Ecol.* 89, 1302–1316. doi: 10.1111/1365-2656.13213

Sheriff, M. J., and Thaler, J. S. (2014). Ecophysiological effects of predation risk; an integration across disciplines. *Oecologia* 176, 607–611. doi: 10.1007/s00442-014-3105-5

Sinclair, A. R. E., Mduma, S., and Brashares, J. S. (2003). Patterns of predation in a diverse predator-prey system. *Nature* 425, 288–290. doi: 10.1038/nature01934

Stephenson, J. F., Perkins, S. E., and Cable, J. (2018). Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies. *J. Anim. Ecol.* 87, 1525–1533. doi: 10.1111/1365-2656.12885

Strauss, A. T., Hite, J. L., Civitello, D. J., Shocket, M. S., Caceres, C. E., and Hall, S. R. (2019). Genotypic variation in parasite avoidance behaviour and other mechanistic, nonlinear components of transmission. *Proc. R. Soc. B Biol. Sci.* 286:20192164. doi: 10.1098/rspb.2019.2164

Sukhdeo, M. V. K. (2012). Where are the parasites in food webs? *Parasit. Vectors* 5:239. doi: 10.1186/1756-3305-5-239

Weinstein, S. B., Buck, J. C., and Young, H. S. (2018a). A landscape of disgust. *Science* 359, 1213–1214. doi: 10.1126/science.aas8694

Weinstein, S. B., Moura, C. W., Mendez, J. F., and Lafferty, K. D. (2018b). Fear of feces? Tradeoffs between disease risk and foraging drive animal activity around raccoon latrines. *Oikos* 127, 927–934. doi: 10.1111/oik.04866

Zander, C. D., Josten, N., Detloff, K. C., Poulin, R., McLaughlin, J. P., and Thieltges, D. W. (2011). Food web including metazoan parasites for a brackish shallow water ecosystem in Germany and Denmark. *Ecology* 92, 2007. doi: 10.1890/11-0374.1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

PERSPECTIVE
published: 10 November 2020
doi: 10.3389/fevo.2020.569319

Mussel Shutdown: Does the Fear of Trematodes Regulate the Functioning of Filter Feeders in Coastal Ecosystems?

Christian Selbach* and Kim N. Mouritsen

Department of Biology, Aquatic Biology, Aarhus University, Aarhus, Denmark

Parasite infections have negative impacts on their hosts. Accordingly, many organisms try to detect and avoid infective parasite stages, leading to changes in host behavior or physiology. Such non-consumptive effects (NCEs) on host traits can have cascading impacts on whole ecosystems but remain largely overlooked. Here, we discuss the potential impacts of the presence of free-living trematode stages on blue mussels *Mytilus edulis*, an important ecosystem engineer in coastal habitats, and highlight the ecological implications of these interactions. Specifically, we discuss how parasite avoidance behavior can regulate the filtration activity of these bivalves in coastal ecosystems and show how even moderate changes in mussel behavior can lead to far-reaching shifts in energy flow. Such processes might be further amplified under future climate change developments, since both parasite abundance and biotic productivity are highly temperature dependent. Overall, we hypothesize that, in addition to their more evident consumptive impacts, trematodes act as cryptic ecosystem engineers by shaping bivalve filtration processes via NCEs. Due to our still very limited understanding of these parasite-mediated processes, we outline key questions for future research directions. We hope this perspective will help encourage new efforts to empirically investigate these fascinating processes that can be crucial regulatory forces in complex ecological systems.

Keywords: ***Mytilus edulis*, ecosystem engineer, non-consumptive effects, ecology of fear, parasite, trematode**

Edited by:
Julia Buck,
University of North Carolina
at Wilmington, United States

Reviewed by:
Jonathan Trevor Vannatta,
Purdue University, United States
David W. Thieltges,
Royal Netherlands Institute for Sea
Research (NIOZ), Netherlands

****Correspondence:***
Christian Selbach
christian.selbach@bios.au.dk

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *03 June 2020*
Accepted: *20 October 2020*
Published: *10 November 2020*

Citation:
Selbach C and Mouritsen KN
(2020) Mussel Shutdown: Does
the Fear of Trematodes Regulate
the Functioning of Filter Feeders
in Coastal Ecosystems?
Front. Ecol. Evol. 8:569319.
doi: 10.3389/fevo.2020.569319

INTRODUCTION

Fear shapes ecological landscapes. Animals have long been known to sense and avoid potential predators via a range of behavioral, physiological, and morphological adaptations (Werner and Peacor, 2003; Creel and Christianson, 2008). Even plants have been shown to employ sophisticated perceptual abilities to detect the presence of herbivores and can induce defense mechanisms, such as leaf closure, under perceived danger (Mescher and De Moraes, 2015). Such risk effects or non-consumptive effects (NCEs) have far-reaching impacts on the interaction between organisms and the structure of whole ecosystems, which can even exceed the direct effects of predation (Ripple and Beschta, 2004; Preisser et al., 2005; Creel and Christianson, 2008; Suraci et al., 2016). For example, the waterborne risk cues released by a crab predator change the grazing behavior of snail species in intertidal rocky shores, which in turn controls the abundance and distribution of brown algae and barnacles, and thus shapes the whole community dynamics of these coastal systems (Trussell et al., 2003). Collectively, these ecological consequences of predator avoidance are referred to as the "ecology of fear" (Brown et al., 1999; Zanette and Clinchy, 2019).

Just like predators, parasites can have a strong negative impact on their host's fitness, i.e., survival and reproductive success (Bush and Clayton, 2018), and just like prey fear and try to avoid their predators, the risk of encountering parasites can induce "fear" or "disgust" in free-living organisms (Buck et al., 2018; Weinstein et al., 2018). Accordingly, hosts have developed a range of parasite and pathogen avoidance strategies and mechanisms, ranging from avoiding interaction with infected conspecifics to evading parasite transmission stages directly when they can be detected, or indirectly via cues related to infection risks, such as potentially contaminated food items (Behringer et al., 2018; Buck et al., 2018). How individuals can detect and avoid parasites depends on a wide variety of factors, including the organism's habitat and life style as well as the parasite's transmission and host finding strategies (see Behringer et al., 2018). The ecological impacts of NCEs of parasites have been shown to be comparable to the far-reaching effects of anti-predator responses (Rohr et al., 2009). However, while the ecology of fear is an integral element of our understanding of predator-prey interactions, host-parasite interactions have so far received considerably less attention in this regard. Although empirical evidence of parasite avoidance behaviors is accumulating, our understanding of these interactions is still very limited and needs to include more ecologically important host-parasite systems, in particular from aquatic environments (Behringer et al., 2018).

In coastal marine communities, bivalves often act as ecosystem engineers, i.e., they modulate the availability of resources in an ecosystem (Jones et al., 1994). Blue mussels (*Mytilus edulis*) form extensive mussel beds along Atlantic coastlines, often covering areas of up to hundreds of hectares. These mussel populations play central ecological roles such as filtering out large amounts of organic matter and creating biogenic reefs, on which a range of organisms depend for shelter, substrate, and foraging (Ragnarsson and Raffaelli, 1999; Bertness, 2007; Commito et al., 2008; Larsson et al., 2017). The two trematode species *Himasthla elongata* and *Renicola roscovita* utilize *M. edulis* as a host. Both parasites share similar life cycles and require the infected mussel to be eaten by shorebirds to mature (**Figure 1A**). Blue mussels become infected via free-swimming dispersal stages, the cercariae, that are emitted from the common periwinkle snail *Littorina littorea* and encyst as metacercariae in mussel tissue (Werding, 1969; **Figure 1B**). Trophically transmitted parasites often directly affect their host's behavior or physiology to facilitate transmission. In the case of *H. elongata*, metacercariae predominantly infect the mussel's foot and mantle tissue, reducing byssal thread production, which likely increases the risk of dislodgement and predation (Lauckner, 1983). *Renicola roscovita* encyst mainly in the gills and palps, decreasing the mussel's filtration and growth abilities (Thieltges, 2006; Stier et al., 2015). Moreover, trematode infections induce pathological responses in *M. edulis*, e.g., a decreased heart rate, highlighting that infected individuals are at an energetic disadvantage compared to uninfected conspecifics (Bakhmet et al., 2017). Overall, trematode infections come at immense fitness costs to the mussels, and they should benefit from avoiding the infective cercariae.

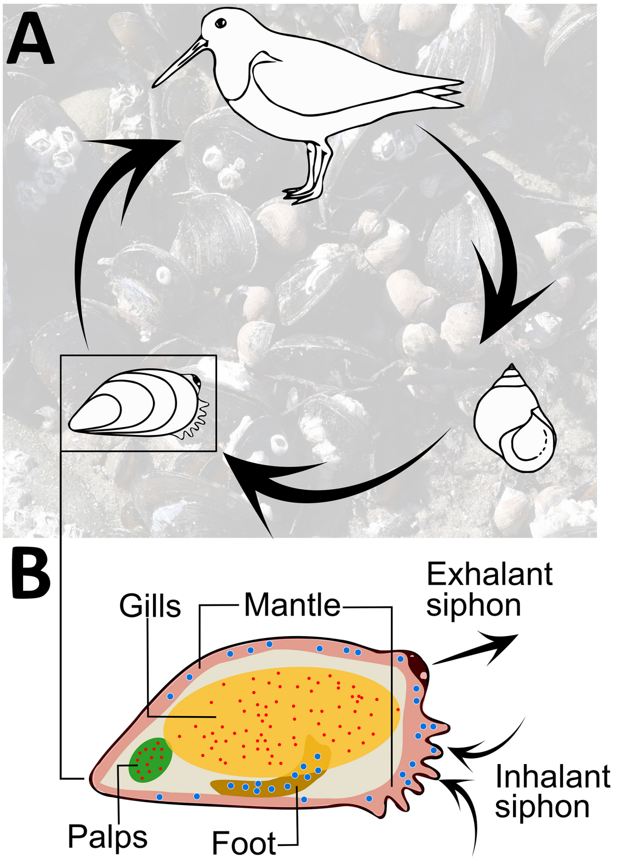

FIGURE 1 | (A) Schematic life cycle of the trematode species *Himasthla elongata* and *Renicola roscovita* that utilize seabirds as final hosts, and periwinkle snails *Littorina littorea* and blue mussels *Mytilus edulis* as first and second intermediate hosts, respectively. Background photo: *M. edulis* and *L. littorea* in a mussel bed during low tide. **(B)** Schematic drawing of the tissues of *M. edulis* that metacercariae of *H. elongata* (large blue dots) and *R. roscovita* (small red dots) typically parasitize; arrows indicate water flow during the mussel's filtration activity. All organisms not drawn to scale.

While more motile hosts groups, such as tadpoles, can evade infectious trematode cercariae via increased swimming activities (Rohr et al., 2009), semi-sessile mussels cannot rely on such escape tactics. Instead, bivalves (cockles) have been reported to strongly react to trematodes in the water by contracting their inhalant siphon and expelling cercariae that were inhaled (Jensen et al., 1999). In laboratory trials, we were able to repeatedly observe a similar behavior in *M. edulis*. When exposed to water containing cercariae of *H. elongata*, mussels rapidly contracted their siphons, before closing their shells completely after a few minutes (**Figure 2**). Such changes in behavior and withdrawing into their shells is a common response to avoid imminent danger from parasites or predators (Dzierżyńska-Białończyk et al., 2019). Although intertidal organisms such as blue mussels are well

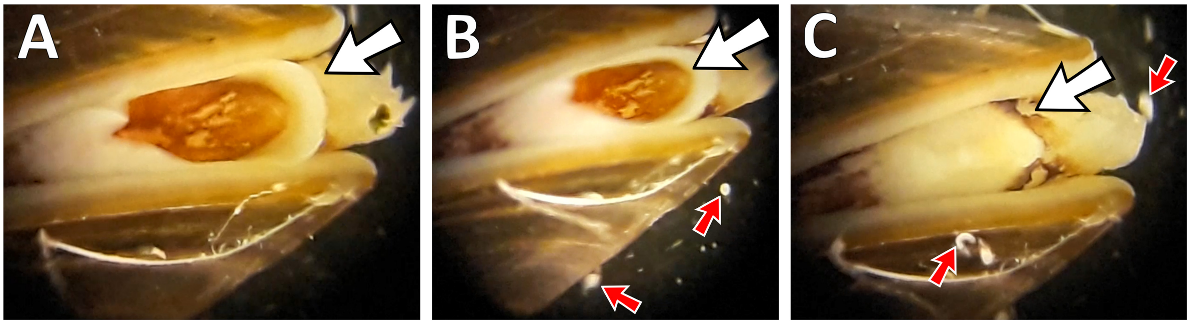

FIGURE 2 | Photo series of *Mytilus edulis* reacting to *Himasthla elongata* cercariae in the water observed under laboratory conditions. **(A)** Mussel filtering with exhalant siphon (white arrow) wide open. **(B)** Cercariae of *H. elongata* (small red arrows) swarming around the mussel. **(C)** Mussel retracting exhalant siphon and ceasing filtration activity.

adapted to rapidly changing environmental conditions and can withstand prolonged periods out of the water, a parasite-induced shell closing prevents mussels from feeding and ventilating when submerged, thereby reducing crucial energy uptake and metabolism (Gosling, 2003). At the same time, such a shutdown of mussel activity results in a reduction in their ability to perform a vital ecosystem function, the removal of organic matter from the pelagic environment. Parasite-NCEs could therefore have severe ecological implications for coastal systems, in particular, under warm conditions when both parasite emission and algal growth peak. However, to what extent these processes take place and could shape ecosystems remains completely overlooked.

Here, we discuss these fundamental interactions and highlight their potential ecological implications based on a mussel-trematode model system. Specifically, our key questions are, (i) how can trematode avoidance behavior impact the filtration ability of bivalves in coastal habitats, and what are the potential ecological consequences, and (ii) since both parasite presence and biotic productivity are temperature dependent, which future developments can be expected under current climate change scenarios? Overall, we hypothesize that trematodes act as cryptic ecosystem engineers by shaping bivalve filtration via NCEs, in addition to their more obvious infection costs. Due to our very limited understanding of these processes, we outline key questions for future research directions.

QUANTIFYING THE ECOLOGICAL COSTS OF MUSSEL SHUTDOWN

Along the North Sea coast, blue mussels form dense beds with thousands of individuals per square meter that have the capacity to filter the water column several times a day and significantly reduce the density of microalgae and smaller mesozooplankton organisms (Prins and Smaal, 1994; Laursen et al., 2010; Dolmer and Stenalt, 2010). Along these coastlines, periwinkle snails from which the infective parasite cercariae emerge live in high density within these blue mussel beds, often grazing directly on and around the mussels (Lauckner, 1984; personal observation; **Figure 1A**). Prevalence of infection in these snail populations shows large temporal and spatial variation but can locally be high and reach more than 50% (Werding, 1969; Lauckner, 1984; Mouritsen, 2017; personal observation). During summer, a single infected snail can release hundreds of cercariae within a few hours (Thieltges and Rick, 2006; personal observation), resulting in large numbers of these infective transmission stages swarming in the water column in search of suitable target hosts (see Morley, 2012). Under such conditions, mussels will regularly come into contact with large numbers of cercariae and be prompted to close their shells to avoid infection with the parasites.

In the western Limfjord, Denmark, a 12,000 m^2 intertidal mussel bed with average densities of 1,000 individuals m^{-2} was shown to clear approximately 37,000 m^3 of water during each tidal cycle (Vismann et al., 2016). After corrections for potential overestimations, the authors conclude that the bivalve bed cleared the available water column in the shallow intertidal zone close to 1 time during a tidal cycle. Since these clearance rates were assessed based on field measurements during the summer months when trematode prevalence and transmission dynamics peak, parasite-induced closure of *M. edulis* and a reduction of their filtration activity are likely an integral but overlooked part of this equation. Although we still lack data to accurately quantify the reduction in mussel clearance rates as a result of parasite avoidance, even moderate NCEs of parasites could translate into strong impacts at the ecosystem level that should be tested in the future.

PARASITES AS CRYPTIC ECOSYSTEM ENGINEERS?

Parasites themselves have been recognized as important ecosystem engineers that modulate the availability of resources to other species via biotic or abiotic changes (Thomas et al., 1999; Pascal et al., 2020). Yet, these estimations of the ecological functions of parasitism are typically only based on post-infection, i.e., consumptive, effects of parasites, which likely grossly underestimates the full impact of these organisms on their environment. In a complex ecosystem, both consumptive and NCEs will take place simultaneously, i.e., a host can already have acquired a number of parasites while trying to avoid further infections. In the case of *M. edulis*, established infections with *R. roscovita* in the gills and palps impair the mussel's filtration ability (Stier et al., 2015), while the presence of cercariae in the environment and subsequent closure of mussels can further limit this activity (see **Figure 2**). This can have potentially interacting or cascading effects on the hosts and their ability to provide valuable ecological functions. Since mussels themselves are important food items to a wide range of keystone predators in the habitat (e.g., crabs and seabirds), the parasite-mediated lower energy uptake and growth rates can be expected to influence the energy flow in coastal systems.

Accordingly, the largely unknown NCEs of parasites are likely playing an important yet still hidden role in the function of these organisms as ecosystem engineers. Moreover, just like NCEs of predators can outweigh the direct ecological impacts of predation (Preisser et al., 2005; Creel and Christianson, 2008; Suraci et al., 2016), this is potentially also true for parasites and pathogens. In the case of blue mussel filtration, the consumptive impact of parasites on the host increases with infection intensity (Thieltges, 2006), i.e., it will only begin to show over time as individual mussels acquire substantial amounts of metacercariae in their tissue. The effects of parasite avoidance behavior, on the other hand, are likely to show immediate effects once significant numbers of trematode cercariae are present in the water and will also affect unparasitized individuals. Overall, both costs of infection and NCEs of parasites have shaped the long co-evolution of intricate host-parasite systems and their interaction (Poulin, 2007; Behringer et al., 2018). For instance, it has been suggested that circadian rhythms of *Mytilus* filtration and the mussels' tendency of to keep their valves closed during daytime has evolved as an anti-predator response (Gnyubkin, 2010). Since cercarial activity in the water typically peaks during daytime, this behavioral adaptation and the ecological implications are very likely shaped by parasite NCEs. To understand the "full" ecosystem engineering potential of host-parasite systems, we must study all effects of parasites on their hosts, and the potential interactions between these factors. This will also advance our understanding of the role of host-parasite systems in changing environments.

TURBID TIMES AHEAD?

Global climate change and ocean warming will have severe and lasting impacts on free-living and parasitic marine biota (Harvell et al., 2002; Marcogliese, 2008; Smale et al., 2019). Although the response of parasites to climate change is complex (see Marcogliese, 2016), the transmission dynamics of trematodes are expected to increase in warmer environments, since higher temperatures can provide favorable conditions for both intermediate and final hosts, allowing longer host-parasite transmission windows (Mas-Coma et al., 2009), and increase the release of infective cercariae into the water (Poulin, 2006; Poulin and Mouritsen, 2006; Studer et al., 2010). Higher levels of trematode infection in bivalves are typically associated with warm water temperatures, e.g., in shallow tidal pools and during summer, indicating that these parasites might benefit from an overall increase in temperature (Thieltges and Rick, 2006). Accordingly, we would expect both the consumptive as well as the NCEs of trematodes on blue mussels to intensify under predicted global warming developments.

At the same time, climate change is altering the distribution and potentially increasing the rate of primary production in the world's oceans (Rabalais et al., 2009; Brown et al., 2010). Under such a scenario, the combination of higher primary production and the parasite-induced reduced filtration activity of bivalves could have implications for the nutrient cycling and food webs in coastal ecosystems. Bivalves might not be able to adequately filter and remove the higher concentration of organic matter from the water column in coastal areas, resulting in increased oxygen depletion and water turbidity, which have been shown for other host-parasite systems (Sánchez et al., 2016).

Under changing climate conditions, even small changes in NCE dynamics could potentially lead to larger cascading impacts, in particular during the warmer summer months. Recent studies have highlighted how trematodes can act as potent ecological engineers and alter the structure and function of communities in intertidal systems under increasing temperatures (Mouritsen et al., 2018). To be able to understand and ultimately predict how ecological systems will react to changing environmental conditions, we therefore need to include the dynamics of parasite NCEs into our assessments.

OPEN QUESTIONS AND FUTURE RESEARCH

Due to our limited knowledge of these important inter-specific interactions, many elements of NCEs on blue mussels remain speculative and will require further investigation. For instance, the exact mechanisms by which bivalves can detect swarming trematode cercariae in the water remain unclear. Blue mussels have been shown to detect and actively react to volatile chemical cues of potential predators in various ways, e.g., by exhibiting lower rates of respiration and activity or seeking refuge in the mussel bed structure (Reimer et al., 1995; Reimer and Tedengren, 1997). Additionally, bivalves and other organisms are capable of sensing and responding to alarm cues from conspecifics that face parasites or predators (Poulin et al., 1999; Kobak and Ryńska, 2014; Dzierżyńska-Białończyk et al., 2019). It should be tested, if mussels are able to react to parasite-induced chemical cues, or if parasite-avoidance behavior is triggered by physical contact with cercariae. Moreover, it is unknown if the mussels' trematode-avoidance strategies constitute species-specific reactions or a generalized response. Since the cercariae of *H. elongata* and *R. roscovita* infect different target tissues and show different infection pathways into their host, they might trigger different parasite avoidance responses. Furthermore, the possible interactions of consumptive and NCEs of parasites on their hosts remain unclear. For example, are already infected mussels more likely to show parasite avoidance behaviors compared to naïve conspecifics, i.e., can mussels learn to avoid parasites (see Behringer et al., 2018)? In addition to *H. elongata* and *R. roscovita*, other trematode species, such as

Proctoeces maculatus, utilize *Mytilus* spp. as a first intermediate host, and as parasitic castrators completely eliminate host fitness (Lauckner, 1983; Buck et al., 2018). It should be expected that the anti-parasite responses of an organism are stronger the higher the fitness costs associated with an infection. Blue mussels offer an attractive model to explore these interactions, due to their role as host to a range of trematodes and other parasites.

In order to assess the ecological impacts of a parasite-induced mussel shutdown, experimental approaches will need to quantify how long mussels close upon encountering cercariae and how this affects clearance and filtration rates under varying conditions, e.g., food availability or host nutritional status (see Hutchings et al., 2000). Ultimately, it remains to be explored to what extent a parasite-mediated reduction in filtration activity translates into changes in nutrient and energy cycling in coastal ecosystems, and how this affects other organisms in coastal habitats. For example, could other organisms consume the organic matter left by mussels, and could parasitism therefore expand a niche for these species? In addition to changes in filtration activity, other impacts of parasite-avoidance behaviors should be explored, such as changes in migration and mussel bed aggregation under perceived threats, similar to behavioral responses of predator-exposed *M. edulis* (Reimer and Tedengren, 1997). Ecological niche shifts due to behavioral and morphological changes have so far been described as a consequence of consumptive effects of trematode infections (e.g., Miura et al., 2006), and it is feasible that NCEs of parasite could have similar impacts. Finally, with regard to future climate changes, a central question is how the dynamics of NCEs of parasites will be affected by changing environmental conditions. In particular, it remains to be tested, if and to what extent changes in water temperature, salinity or resource availability might modulate the mussels' avoidance response to infective parasite stages, and how these processes will impact bivalves and their central ecological functions.

Overall, this range of open questions highlights the possibilities for research projects to answer fundamental ecological questions, using a wide-spread, accessible and relevant mussel-trematode model system for a wide variety of field and laboratory studies.

CONCLUSION

Fear of parasites can shape and moderate organismic interactions and ecological systems. Although we currently still lack data to quantify the exact amount by which NCEs of parasitism do so for most host-parasite groups, there is mounting reason to expect that this is not different from the "ecology of fear" that regulates predator-prey interactions.

Especially in the case of organisms that function as central ecosystem engineers, such as mussels and their trematode parasites, we should seek out to test specific hypotheses to shine more light on these fundamental processes. We understand the open research questions raised in our perspective as potential starting points for this.

We believe the mussel-trematode system discussed here presents an attractive model system for exploring the ecological impacts of parasite avoidance to better understand the processes that shape complex ecological systems. Based on other well-documented cases of parasite and pathogen avoidance and their far-reaching ecological implications, we have no reason to believe that this universal pattern would be different for bivalves and their trematode parasites. Mussels and clams are central ecosystem engineers in many coastal ecosystems and trematodes occur in virtually all aquatic habitats. Therefore, the ecological consequences of NCEs of trematodes on bivalves might be a crucial regulatory force in these habitats that we have long overlooked.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

CS and KNM conceived and wrote the manuscript. Both authors contributed to the article and approved the submitted version.

FUNDING

This project has received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie grant agreement no. 839635 TPOINT.

ACKNOWLEDGMENTS

We thank Janet Koprivnikar for inviting us (on behalf of the other editors) to contribute to this special issue. We also thank Robert Poulin for feedback and remarks on an earlier draft of our manuscript, and two reviewers for their constructive and helpful comments.

REFERENCES

Bakhmet, I., Nikolaev, K., and Levakin, I. (2017). Effect of infection with metacercariae of *Himasthla elongata* (Trematoda: Echinostomatidae) on cardiac activity and growth rate in blue mussels (*Mytilus edulis*) in situ. *J. Sea Res.* 123, 51–54.

Behringer, D. C., Karvonen, A., and Bojko, J. (2018). Parasite avoidance behaviours in aquatic environments. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170202. doi: 10.1098/rstb.2017.0202

Bertness, D. M. (2007). *Atlantic Shorelines: Natural History and Ecology*. Princeton: Princeton University Press.

Brown, C. J., Fulton, E. A., Hobday, A. J., Matear, R. J., Possingham, H. P., Bulman, C., et al. (2010). Effects of climate-driven primary production change on marine food webs: implications for fisheries and conservation. *Glob. Chang. Biol.* 16, 1194–1212. doi: 10.1111/j.1365-2486.2009.02046.x

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.

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

Bush, S. E., and Clayton, D. H. (2018). Anti-parasite behaviour of birds. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170196. doi: 10.1098/rstb.2017.0196

Commito, J. A., Como, S., Grupe, B. M., and Dow, W. E. (2008). Species diversity in the soft-bottom intertidal zone: biogenic structure, sediment, and macrofauna across mussel bed spatial scales. *J. Exp. Mar. Bio. Ecol.* 366, 70–81. doi: 10.1016/j.jembe.2008.07.010

Creel, S., and Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends Ecol. Evol.* 23, 194–201.

Dolmer, P., and Stenalt, E. (2010). The impact of the adult blue mussel (*Mytilus edulis*) population on settling of conspecific larvae. *Aquac. Int.* 18, 3–17.

Dzierżyńska-Białończyk, A., Jermacz, Ł, Zielska, J., and Kobak, J. (2019). What scares a mussel? Changes in valve movement pattern as an immediate response of a byssate bivalve to biotic factors. *Hydrobiologia* 841, 65–77. doi: 10.1007/s10750-019-04007-0

Gnyubkin, V. F. (2010). The circadian rhythms of valve movements in the mussel *Mytilus galloprovincialis*. *Russ. J. Mar. Biol.* 36, 419–428. doi: 10.1134/S1063074010060039

Gosling, E. (2003). *Bivalve Molluscs: Biology, Ecology and Culture*. Oxford: Blackwell Publishing.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., et al. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science* 296, 2158–2162. doi: 10.1126/science.1063699

Hutchings, M. R., Kyriazakis, I., Papachristou, T. G., Gordon, I. J., and Jackson, F. (2000). The herbivores' dilemma: trade-offs between nutrition and parasitism in foraging decisions. *Oecologia* 124, 242–251. doi: 10.1007/s004420000367

Jensen, K. T., Castro, N. F., and Bachelet, G. (1999). Infectivity of *Himasthla* spp. (Trematoda) in cockle (Cerastoderma edule) spat. *J. Mar. Biol. Assoc.* 79, 265–271. doi: 10.1017/S0025315498000290

Jones, C. G., Lawton, J. H., and Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos* 69, 373–386.

Kobak, J., and Ryńska, A. (2014). Environmental factors affecting behavioural responses of an invasive bivalve to conspecific alarm cues. *Anim. Behav.* 96, 177–186. doi: 10.1016/j.anbehav.2014.08.014

Larsson, J., Lind, E. E., Corell, H., Grahn, M., Smolarz, K., and Lönn, M. (2017). Regional genetic differentiation in the blue mussel from the Baltic Sea area. *Estuar. Coast. Shelf Sci.* 195, 98–109. doi: 10.1016/j.ecss.2016.06.016

Lauckner, G. (1983). "Diseases of mollusca: bivalvia," in *Diseases of Marine Animals*, Vol. II, ed. O. Kinne (Hamburg: Biologische Anstalt Helgoland), 477–961.

Lauckner, G. (1984). Impact of trematode parasitism on the fauna of a North Sea tidal flat. *Helgoländer Meeresuntersuchungen* 37, 185–199. doi: 10.1007/BF01989303

Laursen, K., Sand Kristensen, P., and Clausen, P. (2010). Assessment of blue mussel *Mytilus edulis* fisheries and waterbird shellfish-predator management in the Danish Wadden Sea. *Ambio* 39, 476–485. doi: 10.1007/s13280-010-0045-0

Marcogliese, D. J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Rev. Sci. Tech.* 27, 467–484.

Marcogliese, D. J. (2016). The distribution and abundance of parasites in aquatic ecosystems in a changing climate: more than just temperature. *Integr. Comp. Biol.* 56, 611–619. doi: 10.1093/icb/icw036

Mas-Coma, S., Valero, M. A., and Bargues, M. D. (2009). Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. *Vet. Parasitol.* 163, 264–280. doi: 10.1016/j.vetpar.2009.03.024

Mescher, M. C., and De Moraes, C. M. (2015). Role of plant sensory perception in plant-animal interactions. *J. Exp. Bot.* 66, 425–433. doi: 10.1093/jxb/eru414

Miura, O., Kuris, A. M., Torchin, M. E., Hechinger, R. F., and Chiba, S. (2006). Parasites alter host phenotype and may create a new ecological niche for snail hosts. *Proc. R. Soc. B Biol. Sci.* 273, 1323–1328. doi: 10.1098/rspb.2005.3451

Morley, N. J. (2012). *Cercariae* (Platyhelminthes: Trematoda) as neglected components of zooplankton communities in freshwater habitats. *Hydrobiologia* 691, 7–19. doi: 10.1007/s10750-012-1029-9

Mouritsen, K. N. (2017). Periwinkle regulation: parasitism and epibiosis are linked. *Mar. Ecol. Prog. Ser.* 579, 227–231. doi: 10.3354/meps12277

Mouritsen, K. N., Sørensen, M. M., Poulin, R., and Fredensborg, B. L. (2018). Coastal ecosystems on a tipping point: global warming and parasitism combine to alter community structure and function. *Glob. Chang. Biol.* 24, 4340–4356. doi: 10.1111/gcb.14312

Pascal, L., Grémare, A., de Montaudouin, X., Deflandre, B., Romero-Ramirez, A., and Maire, O. (2020). Parasitism in ecosystem engineer species: a key factor controlling marine ecosystem functioning. *J. Anim. Ecol.* 89, 2192–2205. doi: 10.1111/1365-2656.13236

Poulin, R. (2006). Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology* 132, 143–151.

Poulin, R. (2007). *Evolutionary Ecology of Parasites (Second Edition)*. Oxford: Princeton University Press.

Poulin, R., Marcogliese, D. J., and McLaughlin, J. D. (1999). Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *J. Fish Biol.* 55, 47–53. doi: 10.1006/jfbi.1999.0970

Poulin, R., and Mouritsen, K. N. (2006). Climate change, parasitism and the structure of intertidal ecosystems. *J. Helminthol.* 80, 183–191. doi: 10.1079/joh2006341

Preisser, E. L., Bolnick, D. I., and Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* 86, 501–509.

Prins, T. C., and Smaal, A. C. (1994). The role of the blue mussel *Mytilus edulis* in the cycling of nutrients in the Oosterschelde estuary (The Netherlands). *Hydrobiologia* 282, 413–429. doi: 10.1007/BF00024645

Rabalais, N. N., Turner, R. E., Díaz, R. J., and Justić, D. (2009). Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.* 66, 1528–1537. doi: 10.1093/icesjms/fsp047

Ragnarsson, S. Á, and Raffaelli, D. (1999). Effects of the mussel *Mytilus edulis* L. on the invertebrate fauna of sediments. *J. Exp. Mar. Bio. Ecol.* 241, 31–43. doi: 10.1016/S0022-0981(99)00063-5

Reimer, O., Olsson, B., and Tedengren, M. (1995). Growth, physiological rates and behaviour of *Mytilus edulis* exposed to the predator Asterias rubens. *Mar. Freshw. Behav. Phyiol.* 25, 233–244. doi: 10.1080/10236249509378920

Reimer, O., and Tedengren, M. (1997). Predator-induced changes in byssal attachment, aggregation and migration in the blue mussel, *Mytilus edulis*. *Mar. Freshw. Behav. Physiol.* 30, 251–266. doi: 10.1080/10236249709379029

Ripple, W. J., and Beschta, R. L. (2004). Wolves and the ecology of fear: can predation risk structure ecosystems? *Bioscience* 54, 755–766.

Rohr, J. R., Swan, A., Raffel, T. R., and Hudson, P. J. (2009). Parasites, info-disruption, and the ecology of fear. *Oecologia* 159, 447–454. doi: 10.1007/s00442-008-1208-6

Sánchez, M. I., Paredes, I., Lebouvier, M., and Green, A. J. (2016). Functional role of native and invasive filter-feeders, and the effect of parasites: learning from hypersaline ecosystems. *PLoS One* 11:e0161478. doi: 10.1371/journal.pone.0161478

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., et al. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat. Clim. Chang.* 9, 306–312. doi: 10.1038/s41558-019-0412-1

Stier, T., Drent, J., and Thieltges, D. (2015). Trematode infections reduce clearance rates and condition in blue mussels *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 529, 137–144.

Studer, A., Thieltges, D., and Poulin, R. (2010). Parasites and global warming: net effects of temperature on an intertidal host–parasite system. *Mar. Ecol. Prog. Ser.* 415, 11–22. doi: 10.3354/meps08742

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

Thieltges, D. W. (2006). Effect of infection by the metacercarial trematode *Renicola roscovita* on growth in intertidal blue mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 319, 129–134.

Thieltges, D. W., and Rick, J. (2006). Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea: Renicolidae). *Dis. Aquat. Organ.* 73, 63–68.

Thomas, F., Poulin, R., de Meeus, T., Guegan, J.-F., and Renaud, F. (1999). Parasites and ecosystem engineering: what roles could they play? *Oikos* 84, 167–171. doi: 10.2307/3546879

Trussell, G. C., Ewanchuk, P. J., and Bertness, M. D. (2003). Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology* 84, 629–640.

Vismann, B., Wejlemann Holm, M., Davids, J., Dolmer, P., Pedersen, M., Blanda, E., et al. (2016). Field clearance of an intertidal bivalve bed: relative significance of the co-occurring blue mussel *Mytilus edulis* and *Pacific oyster Crassostrea gigas*. *Aquat. Biol.* 25, 107–119. doi: 10.3354/ab00661

Weinstein, S. B., Moura, C. W., Mendez, J. F., and Lafferty, K. D. (2018). Fear of feces? Tradeoffs between disease risk and foraging drive animal activity around raccoon latrines. *Oikos* 127, 927–934. doi: 10.1111/oik.04866

Werding, B. (1969). Morphologie, entwicklung und ökologie digener trematoden-larven der strandschnecke *Littorina littorea*. *Mar. Biol.* 3, 306–333. doi: 10.1007/BF00698861

Werner, E. E., and Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84, 1083–1100.

Zanette, L. Y., and Clinchy, M. (2019). Ecology of fear. *Curr. Biol.* 29, 309–313. doi: 10.1016/j.cub.2019.02.042

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

MINI REVIEW
published: 09 February 2021
doi: 10.3389/fevo.2021.635607

Integrating Concepts of Physiological and Behavioral Resistance to Parasites

*Caroline R. Amoroso**

Department of Biology, University of Virginia, Charlottesville, VA, United States

Conceptual parallels between physiological and behavioral forms of resistance to parasites have led to the development of terminology like "the behavioral immune system" to refer to behaviors that combat parasites. I extend this metaphor by applying findings from research on physiological resistance to generate predictions for the ecology and evolution of behavioral resistance (here, synonymous with avoidance). In certain cases, behavioral resistance may follow similar evolutionary dynamics to physiological resistance. However, more research on the nature of the costs of behavioral resistance is needed, including how parasite transmission mode may be a key determinant of these costs. In addition, "acquiring" behavioral resistance may require specific mechanisms separate from classical forms of conditioning, due to constraints on timing of host learning processes and parasite incubation periods. Given existing literature, behavioral resistance to infectious disease seems more likely to be innate than acquired within the lifetime of an individual, raising new questions about how individual experience could shape anti-parasite behaviors. This review provides a framework for using existing literature on physiological resistance to generate predictions for behavioral resistance, and highlights several important directions for future research based on this comparison.

Keywords: associative learning, behavioral immune system, evolution, host–parasite interactions, parasite avoidance

OPEN ACCESS

Edited by:
Janet Koprivnikar,
Ryerson University, Canada

Reviewed by:
Dana M. Hawley,
Virginia Tech, United States
James Stephen Adelman,
Virginia Tech, United States

***Correspondence:**
Caroline R. Amoroso
cra2z@virginia.edu;
caroline.r.amoroso@gmail.com

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *30 November 2020*
Accepted: *20 January 2021*
Published: *09 February 2021*

Citation:
Amoroso CR (2021) Integrating Concepts of Physiological and Behavioral Resistance to Parasites.
Front. Ecol. Evol. 9:635607.
doi: 10.3389/fevo.2021.635607

INTRODUCTION

Behavior establishes the first interface at which animal hosts and parasites interact (Moore, 2002). Given the parallels between physiological and behavioral forms of resistance to parasites, the concept of the "behavioral immune system" has emerged: the cognitive and behavioral mechanisms for avoiding infectious agents and their cues (Schaller and Duncan, 2007; Schaller and Park, 2011). In other words, through behavioral means, hosts can prevent establishment of parasites analogously to physiological resistance strategies. Does this analogy predict that behavioral resistance should follow the evolutionary and ecological patterns of innate and/or acquired forms of physiological resistance? Although behavioral resistance can also encompass strategies for controlling parasite growth, recovery from infection, and tolerance of infection (Moore, 2002; Hart, 2011; Hawley et al., 2011; Adelman and Hawley, 2017; Townsend et al., 2020), in this review, I explore the parallels between behavioral avoidance of infection and physiological resistance, highlighting potential limitations of acquiring behavioral resistance through individual experience.

DISTINGUISHING PHYSIOLOGICAL AND BEHAVIORAL FORMS OF PARASITE RESISTANCE

First, I establish definitions of physiological and behavioral resistance within the broader framework of host resistance to parasites. I use the term "parasite" for all infectious agents, including micro-organisms usually referred to as "pathogens." Infection of a host by a parasite occurs through a multi-step process that can be broken down into two general steps: (1) contact between host and parasite (host exposure) and (2) establishment of parasite infection in or on the host (host susceptibility) (Combes, 2001; Hall et al., 2017). Exactly how contact is defined will depend upon the transmission mode of the parasite: for a vector-transmitted parasite, it could be the vector biting the host; for a sexually transmitted parasite, it could be deposition of the parasite into the reproductive tract during mating. The host has resistance to the parasite if it can interfere with at least one of these two broad steps along the parasite's path from the previous host. Correspondingly, this two-step process generates two broad categories of resistance: (1) pre-contact resistance, sometimes called "avoidance," which prevents or reduces likelihood of the initial host-parasite contact, and (2) post-contact resistance, which, given contact between host and parasite, interferes with the establishment of parasite infection.

Behaviorals and physiologicals resistance roughly map onto, but do not exactly match, pre- and post-contact resistance. Indeed, behaviors are one form of pre-contact resistance, commonly conceptualized as an avoidance response of animals to a cue of parasite risk (Curtis, 2014). However, pre-contact resistance is not achieved only through behavioral responses: e.g., for pollinator-transmitted parasites of plants, earlier flowering can function as pre-contact resistance (Biere and Antonovics, 1996). Similarly, post-contact resistance does not have to be physiological: e.g., many ectotherms will raise their body temperatures by seeking warm environments to fight parasite infections with a "behavioral fever" (Kluger, 1979). Other behavioral forms of post-contact resistance include grooming (Mooring et al., 2004; Akinyi et al., 2013) and self-medication (Clayton and Wolfe, 1993). Although I do not discuss them thoroughly here, these forms of post-contact behavioral resistance might be expected to have many similarities to post-contact physiological resistance. For the sake of simplicity and drawing connections to the previous literature, I will limit the scope of "behavioral resistance" in this review to pre-contact behavioral resistance, and "physiological resistance" to post-contact physiological resistance.

To compare these two forms of resistance, I also distinguish between innate and acquired physiological resistance, a distinction mostly relevant to vertebrates. Innate resistance refers to the mechanisms that detect and defend against parasites that are present throughout the lifetime of an individual (Beutler, 2004). Acquired resistance refers to the ability to gain resistance in subsequent exposures to a parasite following recovery from an initial exposure, sometimes termed "immunological memory" (Bonilla and Oettgen, 2010). Here, I investigate whether delineating innate and acquired resistance is a useful paradigm to apply to behavior.

EVOLUTION OF INNATE BEHAVIORAL RESISTANCE

Corresponding to innate forms of physiological resistance, behaviors for parasite resistance may be innate and shaped heavily by evolutionary forces. Evidence suggests that parasite avoidance behavior is under the control of genes and therefore likely to evolve. In a knockout experiment in laboratory mice (*Mus musculus*), the oxytocin gene was identified as a central component of the olfactory mechanism female mice used to avoid parasitized males (Kavaliers et al., 2005a). Domestic sheep (*Ovis aries*, Perendale lines) artificially selected for physiological resistance to parasites also avoided parasites in their grazing behavior (Hutchings et al., 2007). In addition, parasite avoidance behavior has been experimentally evolved in *Caenorhabditis elegans* in response to a bacterial parasite, *Serratia marcescens* (Penley and Morran, 2018).

Despite evidence of genetic variation in parasite avoidance behaviors, little is known about the forces that drive their evolution (i.e., allele frequency change). A rich literature has considered theoretical aspects of ecological feedbacks on the evolution of physiological resistance (e.g., Antonovics and Thrall, 1994; Boots and Haraguchi, 1999; Boots et al., 2009). Capturing dynamics of the evolution of physiological resistance involves considering the nature and magnitude of the costs of resistance, and how evolutionary processes are affected by the numbers of hosts and pathogens, in addition to changes in allele frequencies (Antonovics and Thrall, 1994; Boots and Haraguchi, 1999). Identically to models of physiological resistance, theory predicts that if behavioral resistance to a directly transmitted parasite is assumed to carry a fixed cost, resistance is more likely to evolve when costs are low, and resistant and susceptible genotypes can exist in a stable polymorphism under certain conditions (Amoroso and Antonovics, 2020).

However, because behavioral resistance commonly acts before contact between the host and the parasite, the costs of resistance may depend on the transmission mode of the parasite, which defines a contact between the host and the parasite. For example, if social interactions are assumed to be beneficial but also carry a risk of parasite transmission, resistant hosts pay a cost of losing these social interactions, and behavioral resistance (avoidance of conspecifics) is much less likely to evolve (Amoroso and Antonovics, 2020). These conclusions depend on the assumption that the parasite is contact-transmitted, because the costs and benefits of resistance are a function of the host's social behavior. In a social host, resistance could even function as groups avoiding other groups, with costs allocated or shared among group members. Under different transmission modes, the costs could take different forms: e.g., hypothetically, locomotion costs of avoiding habitats with high risk of environmentally transmitted parasites, or reproductive costs of missed mating opportunities when avoiding sexually transmitted parasites. To better understand how behavioral resistance to parasites could evolve, future work will need to conceptualize and measure the associated costs, which may be unique to different parasite transmission modes.

The genetics of coevolution between hosts and parasites have been intensively studied in terms of physiological resistance, generating predictions for coevolutionary dynamics of behavioral resistance. For example, the gene-for-gene model of resistance was originally developed to explain cases in which a plant host's resistance to infection and a parasite's ability to cause disease were each determined by a single genetic locus (Flor, 1956; Frank, 1993). Similar to gene-for-gene dynamics, the host could detect and behaviorally avoid a specific elicitor produced by the parasite, and parasites could evade detection by losing that elicitor. A gene-for-gene model could possibly govern coevolution between *C. elegans*, in which the gene controlling lawn-leaving (avoidance) behavior has been identified (Peng et al., 2018), and its bacterial parasite *Bacillus thuringiensis*, in which the gene has been identified that governs production of a toxin that *C. elegans* detects (Nakad et al., 2016). However, the coevolutionary dynamics of these traits have not yet been examined. In addition, theoretical assessments of coevolutionary processes in mate choice have suggested that avoidance behaviors can influence virulence evolution in sexually transmitted parasites (Ashby and Boots, 2015; Ashby, 2020), and similar predictions could follow for the coevolutionary dynamics of other host avoidance behaviors and parasite transmission modes.

Although hosts could respond to specific cues produced by the parasite, they might instead respond to general cues: a behavioral equivalent to the concept of broad spectrum resistance in plants (e.g., Ke et al., 2017), or cross-immunity of a host to multiple strains of a parasite (Haraguchi and Sasaki, 1997). Parasites are likely to be impossible for hosts to detect directly (Hart, 2011), and therefore selection may be based on detectable yet indirect cues: for example, avoiding feces (Sarabian and MacIntosh, 2015; Amoroso et al., 2017), detecting general signs of disease in conspecifics (Curtis et al., 2004; Paciência et al., 2019), or swatting away insect vectors (Hart and Hart, 1994). If evolved resistance behaviors are general, they could confer resistance to multiple parasites transmitted through the same route, similar to the broader protections offered by the innate immune system. In this way, coevolutionary dynamics between a host and one of its parasites could have ramifications for other parasites transmitted via the same route, possibly resulting in diffuse coevolution (Iwao and Rausher, 1997).

ACQUIRED BEHAVIORAL RESISTANCE

In addition to innate forms of resistance, behaviors for resisting parasites might be acquired in the course of an individual's lifetime, analogous to immunological memory. Evidence supports that animals can learn to avoid predators (reviewed in Griffin et al., 2000), raising the possibility that prior experience of infection with a parasite could induce a novel aversion to the conditions of exposure to that parasite. Classical conditioning research suggests that animals can reliably associate two things when they are separated by a very short time delay, on the order of seconds (Perin, 1943; Renner, 1964). Meanwhile, for most parasites, the incubation period—the time lag between a host being exposed to a parasite and the onset of clinical signs of infection—is on the order of days (Lessler et al., 2009; Azman et al., 2013; Lee et al., 2013; Rudolph et al., 2014). On the short end of this spectrum are parasites with incubation periods of 12 h (e.g., influenza B) (Lessler et al., 2009), and as short as 4 h for some foodborne illnesses (Eley, 1992). Thus, the onset of experienced signs of infection typically occurs too late relative to exposure for a host to associate the two events via classical forms of conditioning, making it exceedingly difficult for hosts to learn to avoid parasite exposure.

However, at least two exceptions exist to the expectation that parasite avoidance is unlikely to be acquired. First, association between exposure to parasites and infection signs would be possible when the host can perceive the parasite's attacks simultaneously with exposure, similar to conditions for learning predator avoidance, such as large, conspicuous ectoparasites like biting flies, whose bites are painful. A series of experiments have shown that both laboratory mice and deer mice (*Peromyscus maniculatus*), upon naïve exposure to biting flies, will avoid the flies by burrowing into the bedding of their cages (Kavaliers et al., 2001, 2003, 2005b). On subsequent exposures, the mice will burrow even if the flies' biting mouthparts have been removed: evidence of a learned avoidance response (Kavaliers et al., 2001). Mice can also learn through observation to avoid the flies with mouthparts removed, without any prior experience of being bitten (Kavaliers et al., 2001, 2003, 2005b). If such avoidance can be learned, it could also have downstream effects on the vector-borne parasites that these conspicuous ectoparasites transmit. It is important to note that in addition to being detectable, a parasite must also induce experienced negative effects (e.g., pain, other clinical signs) in temporal proximity to the infestation (e.g., fly bite). For example, although rock pigeons (*Columba livia*) could detect lice in their feathers, prior experience did not improve rate or efficacy of preening (Villa et al., 2016). The authors suggest that the relatively low virulence and lack of immediate negative fitness effects of lice could explain the absence of a "priming" effect (Villa et al., 2016).

A second exception is a phenomenon called "conditioned taste aversion," the association of taste and odor with gastrointestinal distress such as nausea or vomiting, which can take place over a delay of hours, longer than observed in traditional operant conditioning. In an experiment, an hour or more after ingesting flavored water, mice were injected with a toxin that induced gastric disturbances. On subsequent trials, the mice reliably avoided the flavored water, even after just

TABLE 1 | Several key contrasts between physiological and behavioral resistance.

Physiological resistance	Behavioral resistance
Primarily interferes with infection processes *after* contact between the host and the parasite	Primarily interferes with transmission processes *before* contact between the host and the parasite
Resistance cost often modeled as fixed	Costs may be fixed, social, or some other form that depends on parasite transmission mode
Detection of parasite likely to be on a specific basis	Detection of parasite may commonly be based on general indicators
Can be acquired in the lifetime of an individual	Less likely to be acquired within an individual's lifetime, though learning may shape aspects of behavioral resistance
Individual-level trait	Could function at the group level; groups may avoid other groups

The "Behavioral resistance" column offers future directions for research on this topic.

one experience (Garcia et al., 1966). This phenomenon has since been confirmed in a wide variety of animals, over delays commonly ranging from 1 to 7 h, and up to 24 h (Lin et al., 2017)—long enough to encompass some shorter incubation periods of parasites. Conditioned taste aversion has been extended to associations between specific flavors or odors and digestive discomfort induced specifically by parasite infection. For example, *Drosophila melanogaster* avoided smells present when they ingested a virulent bacterial parasite (Babin et al., 2014), and *Rattus norvegicus domestica* became averse to ingesting flavors following injection with third-stage nematode larvae (Keymer et al., 1983). Taste-conditioned avoidance of parasites has also been demonstrated in *C. elegans* with lawn-leaving assays (Zhang et al., 2005), and has been suggested to be heritable for multiple generations (Moore et al., 2019). Whether learned avoidance behaviors can be transmitted across generations in other host–parasite systems analogously to maternal transmission of antibodies (Grindstaff et al., 2003) deserves further research.

In humans, the improbability of individual learning as an effective strategy for parasite avoidance is underscored by the misattributions of the cause of infectious diseases prior to wide acceptance of germ theory. For example, the widespread belief in the miasma theory of disease—that diseases are acquired through inhaling "bad air"—as recently as the late 19th Century (Halliday, 2001) suggests that for most of our evolutionary history, humans could not reliably associate the particulars of our exposures to infectious agents with symptoms that ensued after a delay. However, human social learning and communication have since proven effective at coordinating widespread avoidance behaviors, including strategies such as hygiene and social distancing (Curtis, 2014; Townsend et al., 2020).

The literature reviewed here suggests that most host behavioral resistance strategies are likely to be innate, rather than acquired through past experience. Furthermore, given the constraints of classical learning processes and typical incubation periods of parasites, not only could acquiring behavioral avoidance of parasites be ineffective, but reliance on learning may lead to incorrect associations between harmless stimuli and experienced signs of infection. Some specialized learning processes, such as imprinting, have even been suggested to cause maladaptive attraction to conspecifics displaying signs of infection with directly transmitted parasites (Stephenson and Reynolds, 2016). Although aversion to parasites is only likely to be acquired in specific circumstances, prior experience could still influence the contexts in which individuals perform behavioral resistance strategies, e.g., if animals associate an innately aversive cue of parasite risk with a specific individual or habitat. Thus, even if behavioral resistance is unlikely to be acquired analogously to immunological memory, future research should consider what role individual experience could play in shaping behavioral resistance.

DISCUSSION

The examples discussed here illustrate that extending the analogy of the "behavioral immune system" can be a useful framework to generate predictions about behavioral resistance to parasites, but should be applied cautiously and with attention to the biology of the processes involved (**Table 1**). A rich literature on physiological resistance to parasites has investigated the population genetics and numerical dynamics that lead to the evolution of different forms of resistance (Boots and Bowers, 2004; Boots et al., 2009). Given that little is known about the specific processes that generate changes in frequency of alleles for behavioral resistance to parasites, expectations from theoretical and empirical research on physiological resistance are a useful starting point from which to advance. This review suggests that evolutionary dynamics of behavioral resistance may be predicted reasonably well by physiological resistance theory under certain assumptions. But more empirical research on the evolutionary dynamics of behavioral resistance is needed, especially on the costs, and the possibility that they may vary with parasite transmission mode. On the other hand, only in particular circumstances it is likely that behavioral resistance to a parasite would be acquired newly in the lifetime of an individual, a major departure from processes of acquired physiological resistance. If behavioral resistance is primarily an innate, evolved response to parasites, understanding the evolutionary—and coevolutionary—processes that have generated behavioral resistance, and whether general principles govern these processes should be central aims in future studies of host behavioral responses to parasites.

I have considered behavioral and physiological resistance separately here, but behavioral resistance precedes physiological resistance in the course of a host's interaction with a single parasite; thus, these two forms of resistance are not independent. Physiological and behavioral resistance are expected to interact with one another. For example, they made trade off, with individuals balancing investment in immune and behavioral defenses (Zylberberg et al., 2013; Zylberberg, 2014). Together, behavioral and physiological resistance constitute a step-wise infection process, in which multiple sequential steps are required (Hall et al., 2017). Not only is a two-step process likely to lead to unique infection genetics, but genes for functionally independent resistance traits can become linked when they are jointly affected by coevolutionary processes (Fenton et al., 2009, 2012). Behavioral and physiological resistance evolution might be expected to interact in many ways, an exciting open direction for future investigation.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This study was supported by NIH (Grant No. R01GM122061), part of the joint NSF-NIH-USDA Ecology and Evolution of Infectious Diseases Program.

ACKNOWLEDGMENTS

I would like to thank Janis Antonovics for providing feedback on the manuscript and for many helpful discussions on this topic.

REFERENCES

Adelman, J. S., and Hawley, D. M. (2017). Tolerance of infection: A role for animal behavior, potential immune mechanisms, and consequences for parasite transmission. *Horm. Behav.* 88, 79–86. doi: 10.1016/j.yhbeh.2016.10.013

Akinyi, M. Y., Tung, J., Jeneby, M., Patel, N. B., Altmann, J., and Alberts, S. C. (2013). Role of grooming in reducing tick load in wild baboons (*Papio cynocephalus*). *Anim. Behav.* 85, 559–568. doi: 10.1016/j.anbehav.2012.12.012

Amoroso, C. R., and Antonovics, J. (2020). Evolution of behavioural resistance in host – pathogen systems. *Biol. Lett.* 16:20200508.

Amoroso, C. R., Frink, A. G., and Nunn, C. L. (2017). Water choice as a counterstrategy to faecally transmitted disease: an experimental study in captive lemurs. *Behaviour* 154, 1239–1258. doi: 10.1163/1568539X-00003466

Antonovics, J., and Thrall, P. H. (1994). The cost of resistance and the maintenance of genetic polymorphism in host-pathogen systems. *Proc. R. Soc. B Biol. Sci.* 257, 105–110.

Ashby, B. (2020). Antagonistic coevolution between hosts and sexually transmitted infections. *Evolution* 74, 43–56. doi: 10.1111/evo.13883

Ashby, B., and Boots, M. (2015). Coevolution of parasite virulence and host mating strategies. *Proc. Natl. Acad. Sci.* 112, 13290–13295. doi: 10.1073/pnas.1508397112

Azman, A. S., Rudolph, K. E., Cummings, D. A. T., Lessler, J., and Tor, E. (2013). The incubation period of cholera: A systematic review supplement. *J. Infect.* 66, 432–438. doi: 10.1016/j.jinf.2012.11.013.The

Babin, A., Kolly, S., Schneider, F., Dolivo, V., Zini, M., and Kawecki, T. J. (2014). Fruit flies learn to avoid odours associated with virulent infection. *Biol. Lett.* 10:20140048.

Beutler, B. (2004). Innate immunity: An overview. *Mol. Immunol.* 40, 845–859. doi: 10.1016/j.molimm.2003.10.005

Biere, A., and Antonovics, J. (1996). Sex-specific costs of resistance to the fungal pathogen Ustilago violacea (Microbotryum violaceum) in Silene alba. *Evolution* 50, 1098–1110. doi: 10.1111/j.1558-5646.1996.tb02350.x

Bonilla, F. A., and Oettgen, H. C. (2010). Adaptive immunity. *J. Allergy Clin. Immunol.* 125, S33–S40. doi: 10.1016/j.jaci.2009.09.017

Boots, M., Best, A., Miller, M. R., and White, A. (2009). The role of ecological feedbacks in the evolution of host defence: What does theory tell us? *Philos. Trans. R. Soc. B Biol. Sci.* 364, 27–36. doi: 10.1098/rstb.2008.0160

Boots, M., and Bowers, R. G. (2004). The evolution of resistance through costly acquired immunity. *Proc. R. Soc. B Biol. Sci.* 271, 715–723. doi: 10.1098/rspb.2003.2655

Boots, M., and Haraguchi, Y. (1999). The evolution of costly resistance in host-parasite systems. *Am. Nat.* 153, 359–370. doi: 10.1086/303181

Clayton, D. H., and Wolfe, D. (1993). The adaptive significance of self-medication. *Trends Ecol. Evol.* 8, 60–63.

Combes, C. (2001). *Parasitism: The Ecology and Evolution of Intimate Interactions*. Chicago: University of Chicago Press.

Curtis, V. A. (2014). Infection-avoidance behaviour in humans and other animals. *Trends Immunol.* 35, 457–464. doi: 10.1016/j.it.2014.08.006

Curtis, V., Aunger, R., and Rabie, T. (2004). Evidence that disgust evolved to protect from risk of disease. *Proc. R. Soc. B Biol. Sci.* 271, S131–S133. doi: 10.1098/rsbl.2003.0144

Eley, A. R. (1992). "Infective bacterial food poisoning," in *Microbial Food Poisoning*, ed. A. R. Eley (Boston, MA: Springer), 15–35.

Fenton, A., Antonovics, J., and Brockhurst, M. A. (2009). Inverse-gene-for-gene infection genetics and coevolutionary dynamics. *Am. Nat.* 174:645087. doi: 10.1086/645087

Fenton, A., Antonovics, J., and Brockhurst, M. A. (2012). Two-step infection processes can lead to coevolution between functionally independent infection and resistance pathways. *Evolution* 66, 2030–2041. doi: 10.1111/j.1558-5646.2012.01578.x

Flor, H. H. (1956). The complementary genic systems in flax and flax rust. *Adv. Genet.* 8, 29–54.

Frank, S. A. (1993). Coevolutionary genetics of plants and pathogens. *Evol. Ecol.* 7, 45–75. doi: 10.1007/BF01237734

Garcia, J., Ervin, F. R., and Koelling, R. A. (1966). Learning with prolonged delay of reinforcement. *Psychon. Sci.* 5, 121–122. doi: 10.3758/BF03328311

Griffin, A. S., Blumstein, D. T., and Evans, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conserv. Biol.* 14, 1317–1326. doi: 10.1046/j.1523-1739.2000.99326.x

Grindstaff, J. L., Brodie, E. D., and Ketterson, E. D. (2003). Immune function across generations: Integrating mechanism and evolutionary process in maternal antibody transmission. *Proc. R. Soc. B Biol. Sci.* 270, 2309–2319. doi: 10.1098/rspb.2003.2485

Hall, M. D., Bento, G., and Ebert, D. (2017). The Evolutionary Consequences of Stepwise Infection Processes. *Trends Ecol. Evol.* 32, 612–623. doi: 10.1016/j.tree.2017.05.009

Halliday, S. (2001). Death and miasma in Victorian London: An obstinate belief. *Br. Med. J.* 323, 1469–1471. doi: 10.1136/bmj.323.7327.1469

Haraguchi, Y., and Sasaki, A. (1997). Evolutionary pattern of intra-host pathogen antigenic drift: effect of corss-reactivity in immune response. *Philos. Trans. R. Soc. B Biol. Sci.* 352, 11–20.

Hart, B., and Hart, L. (1994). Fly switching by Asian elephants: tool use to control parasites. *Anim. Behav.* 48, 35–45. doi: 10.1006/anbe.1994.1209

Hart, B. L. (2011). Behavioural defences in animals against pathogens and parasites: parallels with the pillars of medicine in humans. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 3406–3417. doi: 10.1098/rstb.2011.0092

Hawley, D. M., Etienne, R. S., Ezenwa, V. O., and Jolles, A. E. (2011). Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integr. Comp. Biol.* 51, 528–539. doi: 10.1093/icb/icr062

Hutchings, M. R., Knowler, K. J., McAnulty, R., and McEwan, J. C. (2007). Genetically resistant sheep avoid parasites to a greater extent than do susceptible sheep. *Proc. R. Soc. B Biol. Sci.* 274, 1839–1844. doi: 10.1098/rspb.2007.0398

Iwao, K., and Rausher, M. D. (1997). Evolution of plant resistance to multiple herbivores: Quantifying diffuse coevolution. *Am. Nat.* 149, 316–335. doi: 10.1086/285992

Kavaliers, M., Choleris, E., Ågmo, A., Muglia, L. J., Ogawa, S., and Pfaff, D. W. (2005a). Involvement of the oxytocin gene in the recognition and avoidance of parasitized males by female mice. *Anim. Behav.* 70, 693–702. doi: 10.1016/j.anbehav.2004.12.016

Kavaliers, M., Choleris, E., and Colwell, D. D. (2001). Learning from others to cope with biting flies: social learning of fear-induced conditioned analgesia and active avoidacne. *Behav. Neurosci.* 115, 661–674.

Kavaliers, M., Colwell, D. D., and Choleris, E. (2003). Learning to fear and cope with a natural stressor: Individually and socially acquired corticosterone and avoidance responses to biting flies. *Horm. Behav.* 43, 99–107. doi: 10.1016/S0018-506X(02)00021-1

Kavaliers, M., Colwell, D. D., and Choleris, E. (2005b). Kinship, familiarity and social status modulate social learning about "micropredators" (biting flies) in deer mice. *Behav. Ecol. Sociobiol.* 58, 60–71. doi: 10.1007/s00265-004-0896-0

Ke, Y., Deng, H., and Wang, S. (2017). Advances in understanding broad-spectrum resistance to pathogens in rice. *Plant J.* 90, 738–748. doi: 10.1111/tpj.13438

Keymer, A., Crompton, D. W. T., and Sahakian, B. J. (1983). Parasite-induced learned taste aversion involving Nippostrongylus in rats. *Parasitology* 86, 455–460. doi: 10.1017/S0031182000050642

Kluger, M. J. (1979). Fever in ectotherms: Evolutionary implications. *Integr. Comp. Biol.* 19, 295–304. doi: 10.1093/icb/19.1.295

Lee, R. M., Lessler, J., Lee, R. A., Rudolph, K. E., Reich, N. G., Perl, T. M., et al. (2013). Incubation periods of viral gastroenteritis: A systematic review. *BMC Infect. Dis.* 13:446. doi: 10.1186/1471-2334-13-446

Lessler, J., Reich, N. G., Brookmeyer, R., Perl, T. M., Nelson, K. E., and Cummings, D. A. T. (2009). Incubation period of acute respiratory viral infections: a systematic review. *Lancet Infect. Dis.* 9, 291–300.

Lin, J. Y., Arthurs, J., and Reilly, S. (2017). Conditioned taste aversions: From poisons to pain to drugs of abuse. *Psychon. Bull. Rev.* 24, 335–351. doi: 10.3758/s13423-016-1092-8

Moore, J. (2002). *Parasites and the behavior of animals*. New York: Oxford University Press.

Moore, R. S., Kaletsky, R., and Murphy, C. T. (2019). Piwi/PRG-1 Argonaute and TGF-β Mediate Transgenerational Learned Pathogenic Avoidance. *Cell* 177, 1827.e–1841.e. doi: 10.1016/j.cell.2019.05.024

Mooring, M. S., Blumstein, D. T., and Stoner, C. J. (2004). The evolution of parasite-defence grooming in ungulates. *Biol. J. Linn. Soc.* 81, 17–37. doi: 10.1111/j.1095-8312.2004.00273.x

Nakad, R., Snoek, L. B., Yang, W., Ellendt, S., Schneider, F., Mohr, T. G., et al. (2016). Contrasting invertebrate immune defense behaviors caused by a single gene, the Caenorhabditis elegans neuropeptide receptor gene npr-1. *BMC Genomics* 17:1–20. doi: 10.1186/s12864-016-2603-8

Paciência, F. M. D., Rushmore, J., Chuma, I. S., Lipende, I. F., Caillaud, D., Knauf, S., et al. (2019). Mating avoidance in female olive baboons (Papio anubis) infected by Treponema pallidum. *Sci. Adv.* 5, 1–8. doi: 10.1126/sciadv.aaw9724

Peng, D., Luo, X., Zhang, N., Guo, S., Zheng, J., Chen, L., et al. (2018). Small RNA-mediated Cry toxin silencing allows Bacillus thuringiensis to evade Caenorhabditis elegans avoidance behavioral defenses. *Nucleic Acids Res.* 46, 159–173. doi: 10.1093/nar/gkx959

Penley, M. J., and Morran, L. T. (2018). Host mating system and coevolutionary dynamics shape the evolution of parasite avoidance in Caenorhabditis elegans host populations. *Parasitology* 145, 724–730.

Perin, C. T. (1943). A quantitative investigation of the delay-of-reinforcement gradient. *J. Exp. Psychol.* 32, 37–51.

Renner, K. E. (1964). Delay of reinforcement: a historical review. *Psychol. Bull.* 61, 341–361.

Rudolph, K. E., Lessler, J., Moloney, R. M., Kmush, B., and Cummings, D. A. T. (2014). Review article: Incubation periods of mosquito-borne viral infections: a systematic review. *Am. J. Trop. Med. Hyg.* 90, 882–891. doi: 10.4269/ajtmh.13-0403

Sarabian, C., and MacIntosh, A. J. J. (2015). Hygienic tendencies correlate with low geohelminth infection in free-ranging macaques. *Biol. Lett.* 11:20150757.

Schaller, M., and Duncan, L. A. (2007). "The behavioral immune system: Its evolution and social psychological implications," in *Evolution and the social mind: Evolutionary psychology and social cognition*, eds J. P. Forgas, M. G. Haselton, and W. von Hippel (New York, NY: Psychology Press), 293–307.

Schaller, M., and Park, J. H. (2011). The Behavioral Immune System (and Why It Matters). *Curr. Dir. Psychol. Sci.* 20, 99–103. doi: 10.1177/0963721411402596

Stephenson, J. F., and Reynolds, M. (2016). Imprinting can cause a maladaptive preference for infectious conspecifics. *Biol. Lett.* 12, 9–12. doi: 10.1098/rsbl.2016.0020

Townsend, A. K., Hawley, D. M., Stephenson, J. F., and Williams, K. E. G. (2020). Emerging infectious disease and the challenges of social distancing in human and non-human animals. *Proc. Biol. Sci.* 287:20201039. doi: 10.1098/rspb.2020.1039

Villa, S. M., Campbell, H. E., Bush, S. E., and Clayton, D. H. (2016). Does antiparasite behavior improve with experience? An experimental test of the priming hypothesis. *Behav. Ecol.* 27, 1167–1171. doi: 10.1093/beheco/arw032

Zhang, Y., Lu, H., and Bargmann, C. I. (2005). Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. *Nature* 438, 179–184. doi: 10.1038/nature04216

Zylberberg, M. (2014). Galapagos ground finches balance investment in behavioural and immunological pathogen defences. *IBIS* 156, 615–626. doi: 10.1111/ibi.12165

Zylberberg, M., Klasing, K. C., and Hahn, T. P. (2013). House finches (Carpodacus mexicanus) balance investment in behavioural and immunological defences against pathogens. *Biol. Lett.* 9:856. doi: 10.1098/rsbl.2012.0856

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 11 March 2021
doi: 10.3389/fevo.2021.636099

Alpine Newts (*Ichthyosaura alpestris*) Avoid Habitats Previously Used by Parasite-Exposed Conspecifics

David R. Daversa[1,2,3,4]*, *Andrea Manica*[2], *Héctor Bintanel Cenis*[5], *Pilar Lopez*[6], *Trenton W. J. Garner*[3] *and Jaime Bosch*[6,7,8]

[1] La Kretz Center for California Conservation Science, Institute for the Environment and Sustainability, University of California, Los Angeles, Los Angeles, CA, United States, [2] Department of Zoology, University of Cambridge, Cambridge, United Kingdom, [3] Institute of Zoology, Zoological Society of London, London, United Kingdom, [4] Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, [5] Athmos Sustainability, Zaragoza, Spain, [6] Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain, [7] Research Unit of Biodiversity (CSIC, UO, PA), Gonzalo Gutiérrez Quirós s/n, University of Oviedo, Mieres, Spain, [8] Centro de Investigación, Seguimiento y Evaluación, Parque Nacional de la Sierra de Guadarrama, Rascafría, Spain

Many organisms avoid habitats posing risks of parasitism. Parasites are not generally conspicuous, however, which raises the question of what cues individuals use to detect parasitism risk. Here, we provide evidence in alpine newts (*Ichthyosaura alpestris*) that non-visual cues from parasite-exposed conspecifics inform habitat avoidance. Alpine newts breed in aquatic habitats and occasionally move among adjacent terrestrial habitat during breeding seasons. We completed experiments with newts whereby individuals had access to both habitats, and the aquatic habitats varied in prior occupancy by conspecifics with different histories of exposure to the parasitic skin fungus, *Batrachochytrium dendrobatidis* (*Bd*). Continuous filming of newt activity for 2 days provided little evidence that prior use of aquatic habitats by conspecifics, regardless of their *Bd* exposure history, immediately influenced newt habitat use. However, newts that encountered aquatic habitats used specifically by *Bd*-exposed conspecifics on day 1 spent less time aquatic on day 2, whereas other newts did not alter habitat use. Responses could have been elicited by cues generated by *Bd* stages on the conspecifics or, perhaps more likely, cues emitted by the conspecifics themselves. In either case, these observations suggest that newts use non-visual cues sourced from exposed conspecifics to detect *Bd* risk and that those cues cause newts to avoid aquatic habitats. *Bd* may therefore influence host behavior in early phases of interactions, and possibly before any contact with infectious stages is made, creating potential for non-consumptive effects.

Keywords: infection risk, habitat use, non-lethal effects, anti-parasite behavior, disease ecology, non-consumptive effects

Edited by:
Julia Buck,
University of North Carolina
Wilmington, United States

Reviewed by:
Collin James Horn,
University of Alberta, Canada
Thomas Raffel,
Oakland University, United States

Correspondence:
David R. Daversa
ddaversa@ioes.ucla.edu

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *30 November 2020*
Accepted: *05 February 2021*
Published: *11 March 2021*

Citation:
Daversa DR, Manica A, Bintanel Cenis H, Lopez P, Garner TWJ and Bosch J (2021) Alpine Newts (Ichthyosaura alpestris) Avoid Habitats Previously Used by Parasite-Exposed Conspecifics. Front. Ecol. Evol. 9:636099. doi: 10.3389/fevo.2021.636099

INTRODUCTION

Avoiding habitats containing parasites poses clear benefits. Parasitic infections can be detrimental to individual health and fitness, and in some cases, lead to population collapses (Bosch et al., 2001; Briggs et al., 2010). Free-living organisms can minimize the likelihood of being parasitized by reducing time in habitats posing a risk of infection (Hutchings et al., 2001; Koprivnikar and Penalva, 2015; Daversa et al., 2018a; Mierzejewski et al., 2019). Epidemiologically, this behavior reduces rates of contact with infectious parasite stages, an essential ingredient for infection (Anderson and May, 1981). Similar to predator avoidance, parasite avoidance can affect higher-order ecological processes, exemplified by trophic cascades that restructure entire ecosystems (Buck and Ripple, 2017; Buck et al., 2018). Unlike most predators, however, infective parasite stages are in most cases too small relative to their hosts to visually detect, which raises the question of what cues hosts use to avoid habitats posing a risk of infection.

Many cues that inform decision-making are non-visual and come from conspecifics (Chivers et al., 1999; Jacobsen and Stabell, 2004; Plenderleith et al., 2005). Non-visual chemical cues from conspecifics have known roles in mate choice (Plenderleith et al., 2005; Martín and López, 2006) and predator avoidance (Kats and Dill, 1998), and there is evidence in aquatic organisms that parasite infections alter conspecific chemical cues in ways that elicit avoidance behaviors, such as erratic movement and spatial distancing (Poulin et al., 1999; Behringer et al., 2018; Stephenson et al., 2018). Infected conspecifics may also emit cues generated by the parasite that other hosts conceivably detect. Even in the absence of infection, exposure to parasites can affect how conspecific cues are emitted and interpreted (Folstad et al., 1994; Behringer et al., 2018). For example, parasite exposure alters the development of sexual cues, such as attractive odors (Kavaliers et al., 2003, 2004) and bright coloration (Folstad et al., 1994) that can alter mating decisions. The impacts of parasite exposure on host communication provides reason to predict that non-visual cues sourced from parasite-exposed conspecifics drive hosts to reduce use of habitats where the cues are present. This prediction has received little formal testing.

In this study, we tested whether non-visual cues of the parasitic chytrid fungus, *Batrachochytrium dendrobatidis (Bd),* originating from *Bd*-exposed conspecifics elicit changes in aquatic habitat use in female alpine newts (*Ichthyosaura alpestris*). Alpine newts are prolonged breeders that require extended periods courting and egg-laying in fully aquatic habitats (i.e., lakes and ponds) where *Bd* thrives. *Bd* infects many species of amphibians via microscopic zoospores that are free-living and aquatic (Piotrowski et al., 2004). Infections from *Bd* can cause the disease chytridiomycosis, which is associated with mass mortalities in host populations worldwide (Scheele et al., 2019; Fisher and Garner, 2020). Alpine newts can develop symptomatic disease after contracting infections, but in many cases remain asymptomatic (Miaud et al., 2016; Daversa et al., 2018a). Our previous work suggests that the dynamic nature of newt habitat use may be one explanation for this lack of disease (Daversa et al., 2018a). Alpine newts occasionally move among adjacent terrestrial habitat during breeding seasons (Weddeling et al., 2004; Kopecky et al., 2010; Daversa et al., 2018a). We showed that use of terrestrial habitats by male newts lowers risk of *Bd* infection by reducing both the frequency of exposure to *Bd* zoospores and the growth of *Bd* on infected hosts (Daversa et al., 2018a). Further, male newts spent more time terrestrially after contracting *Bd* infections, especially when infection levels were elevated (Daversa et al., 2018a), illustrating how infection with *Bd* impacts male newt behavior. Here, we built on this work to examine the impact of *Bd* on habitat use of female newts and to test the hypothesis that non-visual cues of *Bd* coming from exposed conspecifics elicit similar increases in terrestrial habitat use.

MATERIALS AND METHODS

Alpine newts are widely distributed in Europe. We studied newts occupying alpine ponds in Central Spain (Guadarrama National Park, 40.85023 N, −3.95442 W) where they were introduced in the 1980's. *Bd* was first detected at the sites in midwife toads (*Alytes obstetricans*) in the late 1990's (Bosch et al., 2001). Newts test positive for *Bd* infections at the sites, but evidence suggests that newts here do not develop chytridiomycosis, and population declines have not been observed (Bosch et al., 2018; Daversa et al., 2018a). Nevertheless, experimental *Bd* exposures in adult alpine newts can cause infection-induced mortality (Miaud et al., 2016). Infections from *Bd* can therefore be costly to newts.

We designed and executed a controlled experiment with female newts to test hypotheses for the role of conspecific cues and *Bd* on newt habitat use:

- Null Hypothesis: Previous use of water by conspecifics, regardless of their recent history of exposure to *Bd*, will not affect the time that newts spend in aquatic habitats.
- Alternative Hypothesis 1: Newts will reduce time in aquatic habitats when the water was previously used by conspecifics generally, irrespective of the *Bd* exposure history of the conspecifics.
- Alternative hypothesis 2: Newts will reduce use of aquatic habitats when the water was previously used specifically by conspecifics recently exposed to *Bd*.

We chose to use female newts as experimental subjects to complement our previous experiments with male newts (Daversa et al., 2018a). We ran the experiment for 2 days during late stages of the alpine newt breeding season (August, 2014).

We captured adult male and female newts from a cluster of ponds in Guadarrama National Park and transported them in coolers to "El Ventorrillo," a biological station located apx. 3 miles away. At the station we housed newts in groups, separated by sex and pond of capture, in 20 L plastic containers containing aged and *Bd*-free spring water. To ensure that all newts started the experiment uninfected, we bathed newts with an antifungal solution (100 μL itraconazole/1 L) for 5 min per day for 4 days (Garner et al., 2009; Daversa et al., 2018a). We confirmed infection-free status of all newts through standard molecular diagnostics and with appropriate positive and negative controls (Boyle et al., 2004; Hyatt et al., 2007; Daversa et al., 2018a). Two days prior to the experiment, we transitioned all females to terrariums with saturated mossy substrate to acclimate them to terrestrial conditions while limiting the likelihood of them changing to a terrestrial phase (Kristín and Gvoždík, 2014).

We generated conspecific cues of *Bd* from male newts because only enough females were collected to use as main experimental subjects. We exposed males individually to *Bd* (1.5 L plastic containers that contained 0.4 L water) after randomly assigning sixty male newts to either a *Bd* exposure group (N = 30) or a control group (N = 30). To generate infections, we used a *Bd* isolate (ID: Pen_Ss9.2 stored in the Imperial Culture Collection, Imperial College London) that was recently isolated from an infected larval fire salamander (*Salamandra salamandra*) in Guadarrama, the same site in which newts were captured. For males in the treatment group, we dosed containers once per day with an inoculate containing 2.0×10^6 – 4.0×10^6 active *Bd* zoospores and repeated the process for 5 days. For males in the control group we administered a daily dose of sterile liquid media following the same protocol. Twenty-four hours following the final exposure we began cohousing males in large plastic tubs filled with aged Bd-free spring water in groups of six unexposed males (unexposed group) or in groups of three unexposed newts and three exposed newts (exposed group). We used water from these large plastic tubs for use in the experiment, as opposed to the water used for exposures, to ensure that water did not contain residual *Bd* zoospores or media remaining from our inoculates. Any *Bd* zoospores in the experimental water would have been those shed by exposed males that contracted infections.

For the experiment we rotated eighteen females through a series of 2 L containers that contained equal parts terrestrial and aquatic habitat. The container sides were opaque and prevented visual cues from being transmitted across replicates. Lids were clear, vented plastic which allowed filming while preventing escapes. We used moistened sphagnum moss overlain on a bed of pebbles as terrestrial habitat. The aquatic portion of the containers contained water from one of three sources to mimic different conspecific cues: (a) uninfected and unused aged tap water used for a control ($N = 6$), (b) water taken from a tank housing conspecifics that were not recently exposed to *Bd* ($N = 6$), and (c) water taken from a tank housing conspecifics that were exposed to *Bd* within 14 days of the experiment ($N = 6$). We fully randomized the layout of containers. The experiment began when we introduced newts at random into the terrestrial side of the containers. We kept all newts in the containers for 24 h (day 1), after which we moved each female into the terrestrial side of the adjacent container and kept them there for another 24 h (day 2). Newts therefore experienced conditions from two different tanks over the course of 2-day experiment.

To quantify habitat use over the 48 h experimental period, we digitally recorded female newts using two webcams (Logitech C310, Newark, CA, United States) positioned above the array of eighteen containers. The webcams were wired to a remote laptop (Dell Inspiron 350) and scheduled to automatically capture one image per minute from 9:00 to 21:00 h using ispy webcam software[1]. Experiment procedures and animal collections were approved by the Consejería de Medio Ambiente of Madrid, Spain (permit reference: 10/130923.9/14) and carried out in accordance with approved guidelines.

From the images we classified the state of females as either aquatic (fully or partially in aquatic habitat) or terrestrial (completely out of aquatic habitat) on a per-minute basis. We considered occupation of the container walls as being terrestrial. We calculated the following variables from these data as measures of daily habitat use: (a) the time of first entry into aquatic habitat (i.e., the chronological sequence number when female state first changed from terrestrial to aquatic), (b) the proportion of time that females occupied aquatic habitat (i.e., the number of images for which female state was aquatic divided by the of total number of images), (c) the total number of transitions between the two habitats, and (d) the mean length of stay in aquatic habitat before moving back on land.

We performed two sets of analyses. For both analyses, we analyzed day 1 and day 2 data separately because newts experienced distinct conditions each day. Further, we did not have the power to run single models that accounted for interactions with experimental day. First, we carried out a 'time-to-event' analysis (i.e., survival analysis) to determine whether previous use of water by conspecifics influenced how quickly newts first entered the aquatic habitat. The response variables comprised (a) the image sequence number when newts were first shown in aquatic habitat and (b) an event status that indicated whether or not newts ever entered the aquatic habitat. We ran Cox proportional hazard models that incorporated the following fixed effects: the presence of cues from conspecifics in general (0 = not present, 1 = present) and the presence of cues from *Bd*-exposed conspecifics in particular (0 = not present, 1 = present), such that:

Day 1 time to entry$\sim$ *all conspecific cues (day 1)* + *Bd-exposed conspecific cues (day 1).*

For the model of day 2 activity, we included the cues encountered on both days as fixed effects, to assess whether aquatic habitat experienced on day 1 had a delayed effect on time to entry into aquatic habitat on day 2, such that:

Day 2 time to entry$\sim$ *all conspecific cues (day 2)* + *Bd-exposed conspecific cues (day 2)* + *all conspecific cues (day 1)* + *Bd-exposed conspecific cues (day 1).*

To assess how well the fixed effects predicted time to first entry into aquatic habitat, we compared models with and without each fixed effect, using likelihood ratio tests as a measure of significance.

For our second analysis we ran generalized linear models (GLMs) to test the influence of cues in aquatic habitats on the three other habitat use variables. In the models for the proportion of time that females occupied aquatic habitat, we used the arcsine-transformed value as the dependent variable and a Gaussian error structure. In the models for habitat transitioning, we used the total number of transitions as the dependent variable and a negative binomial error structure to account for overdispersion of the count data. In the model for the average length of stay in aquatic habitat, we used the log-transformed value to normalize the distribution of the data. We included the same fixed effects in the day 1 and Day 2 models as described for the time-to-event analysis (Day 1: presence of cues from any conspecifics, presence of cues from *Bd*-exposed conspecifics; Day 2 presence of cues from any conspecifics on Day 1 and Day 2, presence of cues from *Bd*-exposed conspecifics on Day 1 and Day 2). In both the day 1 and day 2 analyses we generated sets of candidate models based on different combinations of the fixed effects of the full model (Johnson and Omland, 2004). We ranked the performance of model subsets using Akaike's Information Criterion with

---

[1] www.ispyconnect.com

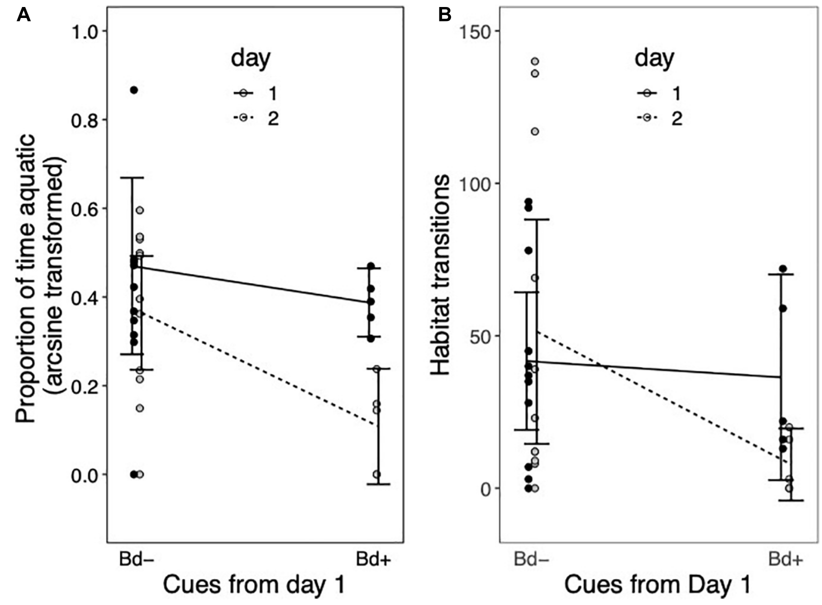

FIGURE 1 | Effects of Day 1 cues on immediate and future aquatic activity. **(A)** The proportion of total time that females spent aquatically (arc-sine transformed) and **(B)** the number of transitions between aquatic and terrestrial habitats on day 1 (black points, solid line) and day 2 (gray points and dashed line) is shown. Newts are classified according to whether they experienced water on Day 1 that was previously used by *Bd*-exposed conspecifics (Bd+) or water with no cues of *Bd* (Bd–). Lines are drawn between the mean value for each class, and error bars denote the 95% confidence intervals.

small sample size correction (AICc) and considered models that ranked within 2 AICc values of the best performing model to be informative (Burnham and Anderson, 2002). We discounted models in which better performing models were nested to avoid the inclusion of uninformative parameters (Arnold, 2010). We performed all analyses in R (R Core Team, 2019). We used the "survival" package (Therneau, 2015) to run the Cox proportional hazard models, "stats" (R Core Team, 2019) and "MASS" (Venables and Ripley, 2002) packages to fit GLMs and the *dredge* function in the "MuMIn" (Bartoñ, 2019) package for model ranking.

RESULTS

We omitted two newts from analyses, leaving a total day 1 and day 2 sample of 16 newts. One newt escaped from a container containing *Bd*-exposed conspecific cues during non-recording hours after day 1. Another died in a container with unexposed conspecific cues during recording hours on day 2. An additional newt never entered the aquatic portion of their container on day 1, leaving the possibility that this newt did not experience Day 1 treatment conditions. Re-analyzing the day 2 data with this newt omitted did not qualitatively change the results (**Supplementary Tables 2,3**).

Habitat use of the remaining sixteen newts was variable across both experimental days (**Figure 1** and **Table 1**). Neither general conspecific cues nor *Bd*-exposed conspecific cues influenced any measures of newt habitat use on the first day (null models performed best in all cases, **Table 2**). Similarly, newt habitat use on day 2 was not influenced by the aquatic conditions encountered on that day (**Table 3**). There was, however, indication that the proportion of time that newts spent aquatic on Day 2 depended on the aquatic conditions encountered on day 1 (**Table 3**). Specifically, newts that encountered water used by *Bd*-exposed conspecifics on day 1 spent proportionally less time in aquatic habitat on Day 2 (**Figure 1A**, effect size ± SE = −0.26 ± 0.11). The aquatic habitats encountered on Day 1 also factored slightly into the number of habitat transitions that newts made on day 2 (effect size = −1.90 ± 0.83, **Table 3**). Newts tended to make fewer transitions between aquatic and terrestrial habitats on day 2 after encountering water from *Bd*-exposed conspecifics on day 1 compared with newts that did not encounter those conditions on day 1 (**Figure 1B**). The aquatic habitats encountered on day 1 and Day 2 did not influence the time that newts first entered aquatic habitat (**Supplementary Table 1**) or the average length of stay in aquatic habitat on either day (**Tables 2**, **3**).

DISCUSSION

Much work has shown that animals make habitat use decisions based on cues of conspecific density and reproductive performance (Danchin et al., 1998; Stamps, 2001; Doligez et al., 2002). Here, we provide evidence that habitat use is also informed by conspecific cues of parasitism. Female newts became more sedentary on land after encountering aquatic habitats previously used by *Bd*-exposed conspecifics. Exactly which cues sourced from *Bd*-exposed conspecifics drove these responses – those produced by conspecifics, those produced by *Bd* stages infecting them, or infectious stages shed into the

TABLE 1 | Summary statistics of the measures of newt habitat use.

	Day 1				Day 2			
Response	**min**	**max**	**mean**	**SE**	**min**	**max**	**mean**	**SE**
Time to first entry into aquatic habitat	1	608	83	41	1	674	129	68
Proportion of time in aquatic habitat	0.00	0.95	0.23	0.06	0.00	0.35	0.12	0.03
Total number of habitat transitions	0.00	94.00	40.06	7.73	0.00	140.00	37.75	12.40

We used automated imaging data collected over the 2-day experiment to calculate the three measures of newt habitat use shown here. The calculations incorporate data from 16 newts. SE, standard error.

TABLE 2 | Factors influencing newt habitat use on Day 1.

Conspecific cues Day 1	Bd cues Day 1	df	ΔAICc	Weight
Proportion of time in aquatic habitat				
		2	0	0.66
	−0.08	3	2.67	0.17
−0.01		3	3.06	0.14
0.03	−0.10	4	6.26	0.03
Number of habitat transitions				
		2	0.00	0.67
0.23		3	2.88	0.16
	−0.14	3	3.01	0.15
0.3483	−0.2801	4	6.28	0.03
Average length of stay in aquatic habitat				
		2	0.00	0.68
−0.03		3	3.07	0.15
	0.00	3	3.08	0.15
−0.04	0.02	4	6.70	0.02

Outputs of multi-model inference testing the factors that influence three measures of newt habitat use on the first day of the experiment: the proportion of time individuals spent in aquatic habitat, the number of transitions made between aquatic and terrestrial habitats, and the average length of stay in aquatic habitats before moving back to land. 'Conspecific cues' denotes containers with aquatic habitats that were previously occupied by conspecifics, regardless of their Bd exposure. 'Bd cues' denotes containers with aquatic habitats that were previously occupied by Bd-exposed conspecifics specifically. Models are listed in order of their Akaike's Information Criteria (AICc) ranking, with the first listed model being the best performing one. Values listed in the factor columns are the model coefficients. Df = degrees of freedom.

TABLE 3 | Factors influencing newt habitat use on Day 2.

Bd cue – Day 1	Bd cue – Day 2	Conspecific cue – Day 1	Conspecific cue – day 2	df	ΔAICc	weight
Proportion of time in aquatic habitat						
−0.26				3	0.00	0.40
−0.20		−0.13		4	1.81	0.16
		−0.22		3	2.31	0.13
−0.26	−0.03			4	3.48	0.07
−0.26			−0.01	4	3.62	0.07
				2	3.97	0.06
	0.02	−0.22		4	5.89	0.02
		−0.22	−0.01	4	5.93	0.02
Number of habitat transitions						
Bd cue – Day 1	**Bd cue – Day 2**	**Conspecific cue – Day 1**	**Conspecific cue – day 2**	**df**	**ΔAICc**	**weight**
−1.89				3	0.00	0.38
				2	1.36	0.19
−1.99			0.47	4	3.24	0.08
		−0.83		3	3.26	0.07
−1.70		−0.37		4	3.42	0.07
−1.91	0.23			4	3.53	0.07
			0.20	3	4.38	0.04
	0.13			3	4.41	0.04
Average length of stay in aquatic habitat						
Bd cue – Day 1	**Bd cue – Day 2**	**Conspecific cue – Day 1**	**Conspecific cue – day 2**	**df**	**ΔAICc**	**weight**
				2.00	0.00	0.29
		−0.34		3.00	0.70	0.20
−0.27				3.00	1.61	0.13
			−0.18	3.00	2.46	0.08
		−0.38	−0.24	4.00	3.04	0.06
	−0.01			3.00	3.08	0.06
−0.15		−0.27		4.00	3.95	0.04
−0.30			−0.22	4.00	4.25	0.03
	−0.02	−0.34		4.00	4.32	0.03
−0.29	−0.08			4.00	5.12	0.02
	0.11		−0.24	4.00	5.90	0.02

Outputs of multi-model inference testing the factors that influence three measures of newt habitat use on the second day of the experiment: the proportion of time individuals spent in aquatic habitat, the number of transitions made between aquatic and terrestrial habitats, and the average length of stay in aquatic habitats before moving back to land. 'Conspecific cues' denotes containers with aquatic habitats that were previously occupied by conspecifics, regardless of their Bd exposure. 'Bd cues' denotes containers with aquatic habitats that were previously occupied by Bd-exposed conspecifics specifically. Models are listed in order of their Akaike's Information Criteria (AICc) ranking, with the first listed model being the best performing one. Values listed in the factor columns are the model coefficients. Only models within 6 AICc values of the best performing model are shown (See ***Supplementary Table 4*** *for full list). Df = degrees of freedom.*

water by conspecifics – remain to be determined. In either case, the responses are evidence that non-visual cues of infection risk sourced from conspecifics can drive newts to alter habitat use. Further, newt increases in terrestrial activity were not immediate, and instead occurred the day after encountering conspecific cues of *Bd*, but still well before *Bd* infections had time to fully develop (which takes at least 4 days, Daversa et al., 2018a). These observations suggest that *Bd* can drive newts to avoid aquatic habitats prior to establishing sustained infections.

The female responses to water used by *B*d-exposed conspecifics strengthens growing evidence that *Bd* imposes costs to occupying aquatic habitats (Miaud et al., 2016; Daversa et al., 2018a). Further, females reduced aquatic activity more proactively than males did in a similar set of experiments (Daversa et al., 2018a), suggesting *Bd* may impose sex specific costs of aquatic activity. For males, there is a premium on spending time in aquatic habitat because females are the choosy sex in terms of mating. Mating pressures may drive males to take more risks in the water before heading for land than females, potentially leading to higher rates of parasitic infection in males (Raffel, 2006).

Bd-imposed costs of aquatic activity present clear trade-offs with breeding and other fitness-related aquatic activities (e.g., feeding, predator evasion) that may shape newt decision-making. Although we intentionally ran our experiments during late stages of the breeding season, the sustained aquatic activity exhibited by females that did not encounter cues of *Bd* suggests that they still had incentive to use aquatic habitats during later breeding stages. Achieving a mating does not ensure fertility for female alpine newts, and many mate multiple times over the course of a breeding season (Garner and Schmidt, 2003; Hoeck and Garner, 2007). Avoidance of aquatic habitats carrying cues of *Bd* clearly conflicts with these mating patterns and potentially compromises fitness. Alternatively, reduced time aquatic habitats could have resulted from females waiting for males posing reduced infection risk, and this increased selectivity could actually increase fitness. The impact of *Bd* cues on female decision-making may also depend on their infection status, which could not be tested here. Understanding how newts allocate time in aquatic versus terrestrial habitat may be achieved, at least in part, from the perspective of trade-offs between reproduction and parasitism.

Even subtle differences in the timing of newt responses to *Bd* may affect their broader ecological consequences (Daversa et al., 2021). *Bd* could conceivably drive population declines in newts non-lethally and non-consumptively through costs to fecundity if the female reductions in aquatic activity observed here are sustained for extended periods of time. Epidemiologically, however, newt avoidance of water with conspecific cues of *Bd* should have negative impacts on the parasite because newt terrestrial activity reduces their likelihood of contracting and maintaining infections (Daversa et al., 2018a). If female reductions in aquatic activity prevent infections from developing, then female terrestriality should drive down infection prevalence and spread, similar to how wildlife migration can reduce infection prevalence and spread by allowing animals to "escape" infection (Altizer et al., 2011; Daversa et al., 2017). Expanding our design to field enclosures with multiple discrete aquatic habitats could gain insight into how female avoidance of conspecific cues of *Bd* in water alter how *Bd* persists and spread over the landscape. Tracking animals over longer time periods than we did would also be useful for clarifying how reductions in aquatic activity impact the course of *Bd* infections.

The specific mechanisms driving females to become more terrestrial remain to be determined, though our experiments favor certain hypotheses. Visual cues from conspecifics were clearly not involved because conspecifics were never present during the experiment. We cannot rule out the possibility that newts responded to active *Bd* zoospores shed into the water previously from *Bd*-exposed conspecifics, or very early stages of infection from those zoospores. However, the low infection burdens that newts typically acquire (Miaud et al., 2016; Daversa et al., 2018a) suggest that zoospore concentrations in water previously used by *Bd*-exposed conspecifics were low. We hypothesize that newt responses were likely elicited by compositional changes in cues emitted by *Bd*-exposed conspecifics, such as pheromones, which newts use to communicate for mating, orientation, and likely other activities (Joly and Miaud, 1993; Denoël and Doellen, 2010; Treer et al., 2013). Although the potential for *Bd* exposure to influence the composition of chemical cues of amphibians has not been tested previously, support for our hypothesis comes from evidence for a chemosensory impact in other aquatic systems (Poulin et al., 1999; Stephenson et al., 2015; Behringer et al., 2018). Experimental data on fish, for example, indicate that individuals avoid conspecifics (Stephenson et al., 2018) and exhibit defensive movements (Poulin et al., 1999) in response to infected conspecifics but not to the parasites themselves. Alternatively, females may have responded to cues of stress left by *Bd*-exposed conspecifics (Gabor et al., 2013; Auld et al., 2014; Nadler et al., 2020). These putative mechanisms could be tested by sampling both aquatic habitats and conspecifics for pheromones, corticosterone, and *Bd* zoospores. Adding treatments to our design that, for example, expose newts directly to *Bd* zoospores in water or to cues from conspecifics facing with other forms of stress (e.g., predator-induced) could disentangle the specific mechanisms driving newt responses to water previously used by *Bd*-exposed conspecifics.

Regardless of the mechanisms involved, our findings build on the evidence base that parasites play a key role in shaping amphibian habitat use (Kriger and Hero, 2007; Raffel et al., 2015; Daversa et al., 2018a,b). This argument is not new. Anecdotes of amphibians going terrestrial in response to parasites, such as leeches, date back to the 1970's (Gill, 1978), and these anecdotes have since been backed by systematic surveillance (Raffel, 2006). This collective body of work leads to an interesting prediction that terrestrial activity may be a general adaptive response for minimizing infection risk. Future empirical work on *Bd* may benefit from more emphasis toward infection dynamics during terrestrial phases of amphibians, which to date remain less studied than infection dynamics in fully aquatic habitats. More broadly, this work points to non-visual cues as a source of information driving parasite avoidance.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Consejería de Medio Ambiente, Madrid, Spain.

AUTHOR CONTRIBUTIONS

DD formulated the hypothesis and wrote the initial manuscript, which was revised according to the comments of AM, TG, HB, and JB. DD, JB, PL, and HB designed the experiments. DD and HB executed the experiments. DD and AM performed the data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by Research England, the Cambridge Trusts, and a grant from the Natural Environment Research Council UK (NE/N009800/1).

ACKNOWLEDGMENTS

We thank the Cambridge Trusts, Research England, and NERC for funding this study, the personnel of Guadarrama mountain national park for supporting the collection of newts; Jose Martin for arranging space for experimental setups, Matthew C. Fisher for culturing *Bd* isolates; Gloria Lopez for assisting with the construction of experimental containers and the other researchers at "El Ventorrillo" biological station for their general support; and the two reviewers for the helpful comments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.636099/full#supplementary-material

REFERENCES

Altizer, S., Bartel, R., and Han, B. A. (2011). Animal migration and infectious disease risk. *Science* 331, 296–302. doi: 10.1126/science.1194694

Anderson, R. M., and May, R. M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 291, 452–491.

Arnold, T. W. (2010). Uninformative parameters and model selection using Akaike's information criterion. *J. Wildl. Manage.* 74, 1175–1178. doi: 10.2193/2009-367

Auld, S. K. J. R., Hall, S. R., Housley Ochs, J., Sebastian, M., and Duffy, M. A. (2014). Predators and patterns of within-host growth can mediate both among-host competition and evolution of transmission potential of parasites. *Am. Nat.* 184, S77–S90. doi: 10.1086/676927

Bartoñ, K. (2019). *MuMIn: Multi-Model Inference*. Available online at: https://CRAN.R-project.org/package=MuMIn (accessed 12, September 2020).

Behringer, D. C., Karvonen, A., and Bojko, J. (2018). Parasite avoidance behaviours in aquatic environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 373:20170202. doi: 10.1098/rstb.2017.0202

Bosch, J., Martínez-Solano, I., and García-París, M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biol. Conserv.* 97, 331–337. doi: 10.1016/s0006-3207(00)00132-4

Bosch, J., Fernández-Beaskoetxea, S., Garner, T. W. J., and Carrascal, L. M. (2018). Long-term monitoring of an amphibian community after a climate change- and infectious disease-driven species extirpation. *Glob. Change Biol.* 24: 2622–2632. doi: 10.1111/gcb.14092

Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T., and Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Organ.* 60, 141–148. doi: 10.3354/dao060141

Briggs, C. J., Knapp, R. A., and Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Natl. Acad. Sci. U. S. A.* 107, 9695–9700. doi: 10.1073/pnas.0912886107

Buck, J. C., and Ripple, W. J. (2017). Infectious agents trigger trophic cascades. *Trends Ecol. Evol.* 32, 681–694. doi: 10.1016/j.tree.2017.06.009

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

Burnham, K. P., and Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. New York, NY: Springer.

Chivers, D. P., Kiesecker, J. M., Wildy, E. L., Belden, L. K., Kats, L. B., and Blaustein, A. R. (1999). Avoidance response of post-metamorphic anurans to cues of injured conspecifics and predators. *J. Herpetol.* 33:472–476. doi: 10.2307/1565645

Danchin, E., Boulinier, T., and Massot, M. (1998). Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology* 79, 2415–2428. doi: 10.1890/0012-9658(1998)079[2415:crsabh]2.0.co;2

Daversa, D. R., Fenton, A., Dell, A. I., Garner, T. W. J., and Manica, A. (2017). Infections on the move: how transient phases of host movement influence disease spread. *Proc. Biol. Sci.* 284:20171807. doi: 10.1098/rspb.2017.1807

Daversa, D. R., Hechinger, R. F., Madin, E., Fenton, A., Dell, A. I., Ritchie, E. G., et al. (2021). Broadening the ecology of fear: non-lethal effects arise from diverse responses to predation and parasitism. *Proc. R. Soc*. B.28820202966. doi: 10.1098/rspb.2020.2966

Daversa, D. R., Manica, A., Bosch, J., Jolles, J. W., and Garner, T. W. J. (2018a). Routine habitat switching alters the likelihood and persistence of infection with a pathogenic parasite. *Funct. Ecol.* 32, 1262–1270. doi: 10.1111/1365-2435.13038

Daversa, D. R., Monsalve-Carcaño, C., Carrascal, L. M., and Bosch, J. (2018b). Seasonal migrations, body temperature fluctuations, and infection dynamics in adult amphibians. *PeerJ* 6:e4698. doi: 10.7717/peerj.4698

Denoël, M., and Doellen, J. (2010). Displaying in the dark: light-dependent alternative mating tactics in the alpine newt. *Behav. Ecol. Sociobiol.* 64, 1171–1177. doi: 10.1007/s00265-010-0933-0

Doligez, B., Danchin, E., and Clobert, J. (2002). Public information and breeding habitat selection in a wild bird population. *Science* 297, 1168–1170. doi: 10.1126/science.1072838

Fisher, M. C., and Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nat. Rev. Microbiol.* doi: 10.1038/s41579-020-0335-x

Folstad, I., Hope, A. M., Karter, A., and Skorping, A. (1994). Sexually selected color in male sticklebacks: a signal of both parasite exposure and parasite resistance? *Oikos* 69, 511–515. doi: 10.2307/3545863

Gabor, C. R., Fisher, M. C., and Bosch, J. (2013). A non-invasive stress assay shows that tadpole populations infected with Batrachochytrium dendrobatidis have elevated corticosterone levels. *PLoS One* 8:e56054. doi: 10.1371/journal.pone.0056054

Garner, T. W., and Schmidt, B. R. (2003). Relatedness, body size and paternity in the alpine newt, *Triturus* alpestris. *Proc. Biol. Sci.* 270, 619–624. doi: 10.1098/rspb.2002.2284

Garner, T. W. J., Garcia, G., Carroll, B., and Fisher, M. C. (2009). Using itraconazole to clear Batrachochytrium dendrobatidis infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. *Dis. Aquat. Organ.* 83, 257–260. doi: 10.3354/dao02008

Gill, D. E. (1978). The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Ecol. Monogr.* 48, 145–166. doi: 10.2307/2937297

Hoeck, P. E., and Garner, T. W. J. (2007). Female alpine newts (*Triturus* alpestris) mate initially with males signalling fertility benefits. *Biol. J. Linn. Soc.* 91, 483–491. doi: 10.1111/j.1095-8312.2007.00813.x

Hutchings, M. R., Gordon, I. J., Kyriazakis, I., and Jackson, F. (2001). Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake rate of forage and parasitism in subsequent foraging decisions. *Anim. Behav.* 62, 955–964. doi: 10.1006/anbe.2001.1837

Hyatt, A. D., Boyle, D. G., Olsen, V., Boyle, D. B., Berger, L., Obendorf, D., et al. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* 73, 175–192. doi: 10.3354/dao073175

Jacobsen, H. P., and Stabell, O. B. (2004). Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. *Oikos* 104, 43–50. doi: 10.1111/j.0030-1299.2004.12369.x

Johnson, J. B., and Omland, K. S. (2004). Model selection in ecology and evolution. *Trends Ecol. Evol.* 19, 101–108. doi: 10.1016/j.tree.2003.10.013

Joly, P., and Miaud, C. (1993). How does a newt find its pond? The role of chemical cues in migrating newts. *Ethol. Ecol. Evol.* 5, 447–455.

Kats, L. B., and Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5, 361–394. doi: 10.1080/11956860.1998.11682468

Kavaliers, M., Choleris, E., Ågmo, A., and Pfaff, D. W. (2004). Olfactory-mediated parasite recognition and avoidance: linking genes to behavior. *Horm. Behav.* 46, 272–283. doi: 10.1016/j.yhbeh.2004.03.005

Kavaliers, M., Colwell, D. D., Braun, W. J., and Choleris, E. (2003). Brief exposure to the odour of a parasitized male alters the subsequent mate odour responses of female mice. *Anim. Behav.* 65, 59–68. doi: 10.1006/anbe.2002.2043

Kopecky, O., Vojar, J., and Denoël, M. (2010). Movements of alpine newts (Mesotriton alpestris) between small aquatic habitats (ruts) during the breeding season. *Amphib. Reptil.* 31, 109–116. doi: 10.1163/156853810790457821

Koprivnikar, J., and Penalva, L. (2015). Lesser of two evils? Foraging choices in response to threats of predation and parasitism. *PLoS One* 10:e0116569. doi: 10.1371/journal.pone.0116569

Kriger, K. M., and Hero, J.-M. (2007). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Divers. Distrib.* 13, 781–788. doi: 10.1111/j.1472-4642.2007.00394.x

Kristín, P., and Gvoždík, L. (2014). Aquatic-to-terrestrial habitat shift reduces energy expenditure in newts. *J. Exp. Zool. A Ecol. Genet. Physiol.* 321, 183–188. doi: 10.1002/jez.1849

Martín, J., and López, P. (2006). Links between male quality, male chemical signals, and female mate choice in Iberian Rock Lizards. *Funct. Ecol.* 20, 1087–1096. doi: 10.1111/j.1365-2435.2006.01183.x

Miaud, C., Dejean, T., Savard, K., Millery-Vigues, A., Valentini, A., Curt Grand Gaudin, N., et al. (2016). Invasive North American bullfrogs transmit lethal fungus *Batrachochytrium dendrobatidis* infections to native amphibian host species. *Biol. Invasions* 18, 2299–2308. doi: 10.1007/s10530-016-1161-y

Mierzejewski, M., Horn, C. J., and Luong, L. T. (2019). Ecology of fear: environment-dependent parasite avoidance among ovipositing drosophila. *Parasitology* 146, 1564–1570. doi: 10.1017/s0031182019000854

Nadler, L. E., Bengston, E., Eliason, E. J., Hassibi, C., Helland-Riise, S. H., Johansen, I. B., et al. (2020). A brain-infecting parasite impacts host metabolism both during exposure and after infection is established. *Funct. Ecol* doi: 10.1111/1365-2435.13695 n/a.

Piotrowski, J. S., Annis, S. L., and Longcore, J. E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96, 9–15. doi: 10.2307/3761981

Plenderleith, M., van Oosterhout, C., Robinson, R. L., and Turner, G. F. (2005). Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biol. Lett.* 1, 411–414. doi: 10.1098/rsbl.2005.0355

Poulin, R., Marcogliese, D. J., and McLaughlin, J. D. (1999). Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *J. Fish Biol.* 55, 47–53. doi: 10.1111/j.1095-8649.1999.tb00655.x

Raffel, T. (2006). *Causes and Consequences of Seasonal Dynamics in the Parasite Community of Red-Spotted Newts (Notophthalmus viridescens).*

Raffel, T. R., Halstead, N. T., McMahon, T. A., Davis, A. K., and Rohr, J. R. (2015). Temperature variability and moisture synergistically interact to exacerbate an epizootic disease. *Proc. Biol. Sci.* 282:20142039. doi: 10.1098/rspb.2014.2039

R Core Team (2019). *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., et al. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363, 1459–1463. doi: 10.1126/science.aav0379

Stamps, J. (2001). "Habitat selection by dispersers: integrating proximate and ultimate approaches," in *Dispersal*, eds J. Clobert., E. Danchin, A. Dhondt, and J. Nichols (Oxford: University of Oxford Press), 230–242.

Stephenson, J. F., Perkins, S. E., and Cable, J. (2018). Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies. *J. Anim. Ecol.* 87, 1525–1533. doi: 10.1111/1365-2656.12885

Stephenson, J. F., Van Oosterhout, C., Mohammed, R. S., and Cable, J. (2015). Parasites of Trinidadian guppies: evidence for sex-and age-specific trait-mediated indirect effects of predators. *Ecology* 96, 489–498. doi: 10.1890/14-0495.1

Therneau, T. M. (2015). *A Package for Survival Analysis in R.* Available online at: https://CRAN.R-project.org/package=survival (accessed February 4, 2021).

Treer, D., Van Bocxlaer, I., Matthijs, S., Du Four, D., Janssenswillen, S., Willaert, B., et al. (2013). Love is blind: indiscriminate female mating responses to male courtship pheromones in newts (Salamandridae). *PLoS One* 8:e56538. doi: 10.1371/journal.pone.0056538

Venables, W. N., and Ripley, B. D. (2002). *Modern Applied Statistics with S.* New York, NY: Springer.

Weddeling, K., Hachtel, M., Sander, U., and Tarkhnishvili, D. (2004). Bias in estimation of newt population size: a field study at five ponds using drift fences, pitfalls and funnel traps. *Herpetol. J.* 14, 1–8.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

PERSPECTIVE
published: 12 March 2021
doi: 10.3389/fevo.2021.634754

Parasite-Modified Chemical Communication: Implications for Aquatic Community Dynamics

Olwyn C. Friesen and Jillian T. Detwiler*

Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Chemical communication within an aquatic environment creates an intricate signaling web that provides species with information about their surroundings. Signaling molecules, like oxylipins, mediate a multitude of interactions between free-living members of a community including non-consumptive effects by predators. Parasites are another source of signaling molecules in aquatic communities and contribute directly by synthesizing them or indirectly by manipulating host chemical cues. If chemical cues of infected hosts are altered, then non-consumptive interactions between other members of the community may also be affected. Different cues from infected hosts may alter behaviors in other individuals related to foraging, competition, and defense priming. Here, we discuss how parasites could modify host chemical cues, which may have far reaching consequences for other community members and the ecosystem. We discuss how the modification of signaling molecules by parasites may also represent a mechanism for parasite-modified behavior within some systems and provide a mechanism for non-consumptive effects of parasites. Further, we propose a host-parasite system that could be used to investigate some key, unanswered questions regarding the relationship between chemical cues, parasite-modified behavior, and non-consumptive effects. We explain how trematode-gastropod systems can be used to test whether there are alterations in the diversity and amounts of signaling molecules available, and if habitat use, immune function, and behavior of other individuals and species are affected. Finally, we argue that changes to pathway crosstalk by parasites within communities may have broad ecological implications.

Keywords: chemical communication, non-consumptive interactions, community dynamics, oxylipins, signaling web, infochemicals, volatile organic compounds, non-consumptive effects

Edited by:
Lien Luong,
University of Alberta, Canada

Reviewed by:
Brandon Parker Ruehle,
University of Otago, New Zealand
Iain James Gordon,
Australian National University,
Australia

****Correspondence:***
Olwyn C. Friesen
olwynfriesen@gmail.com

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *28 November 2020*
Accepted: *19 February 2021*
Published: *12 March 2021*

Citation:
Friesen OC and Detwiler JT
(2021) Parasite-Modified Chemical
Communication: Implications
for Aquatic Community Dynamics.
Front. Ecol. Evol. 9:634754.
doi: 10.3389/fevo.2021.634754

INTRODUCTION

Species obtain important information about their surroundings through intricate signaling webs created by chemical cues (Brönmark and Hansson, 2000; van Poecke and Dicke, 2004; Vos et al., 2006). Cues can have a variety of functions including alarm cues, chemical defense, and sex pheromones, that impact the behavior and physiology of organisms receiving these signals (Kats and Dill, 1998; Kaupp et al., 2006; Fink, 2007; Pohnert et al., 2007; Kita et al., 2010). Aquatic organisms respond to minute concentrations of chemical cues within this environment (Dicke and Sabelis, 1988; Brönmark and Hansson, 2000; Vos et al., 2006). Gradual changes in chemical cue diversity and concentration throughout an ecosystem relays important information to species, creating a network or signaling web that can be used by community members and influence community dynamics (van Poecke and Dicke, 2004; Vos et al., 2006; Fink, 2007; Brönmark and Hansson, 2012). As a result, many interactions, such as predator avoidance, and parasite transmission are influenced by cues produced in the community (Kats and Dill, 1998; Burks and Lodge, 2002; Fink, 2007; Saha et al., 2019).

Within the aquatic community, individuals of other species may also "eavesdrop" on this communication to obtain additional information about their environment (Baldwin et al., 2006; Vos et al., 2006). If predators and parasites can perceive cues through all of the chemicals found within the aquatic system, or "ambient noise," they can take advantage of chemical crosstalk to increase depredation and transmission (Schultz and Appel, 2004; Baldwin et al., 2006; Brönmark and Hansson, 2012). For example, eavesdropping has been shown to facilitate tritrophic interactions in aquatic communities. Plants released oxylipins and other VOCs as they were being consumed by herbivores, which attracted carnivores and parasitoids that subsequently reduced herbivore performance (Martin et al., 2019; Kergunteuil et al., 2020).

Conversely, if a species is able to perceive an increased risk of predation or parasitism through the perception of cues released by other individuals, they may adopt strategies to reduce this risk (Buck et al., 2018; Weinstein et al., 2018). Indeed, chemical cues are important mediators of non-consumptive effects (NCEs) in predator-prey relationships (Ferrari et al., 2010; Hill and Weissburg, 2013). Response to predator cues leads to alterations in behavior and physiology that reduce an individual's risk of predation (Weissburg and Beauvais, 2015; Hermann and Landis, 2017). Relatively less is understood about the role of chemical cues in mediating NCEs of parasites and parasite-host interactions (Fink, 2007; Saha et al., 2019). More work is needed to determine whether parasites perceive and use crosstalk in their non-consumptive interactions with their hosts and other members of the aquatic community.

OXYLIPIN CHEMICAL CUES MEDIATE COMMUNITY INTERACTIONS

Organisms release species-specific chemical emissions, representing a unique cocktail of cues, including fatty acids, amino acids, nucleotides, and other volatile organic compounds (VOCs) (Brönmark and Hansson, 2000; Pohnert, 2002; Fink, 2007). One important group of VOCs are oxylipins, or oxygenated metabolites of fatty acids, (de Petrocellis and di Marzo, 1994; Baldwin et al., 2006) that have essential roles in normal physiology, stress, and immune response of a wide breadth of taxa, from plants to animals (Stanley-Samuelson, 1994; Fink, 2007; Caldwell, 2009; Stanley, 2014; Gabbs et al., 2015). Each oxylipin cocktail carries complex information to the receiver, with the specificity of the mixture being key to dictating the response by the receiver rather than a single oxylipin alone (Metcalf and Kogan, 1987; Wendel and Jüttner, 1996; Fink, 2007). For example, mixtures containing eicosanoids, oxylipins formed from C20:C22 fatty acids, affect the physiology of invertebrates, including the neuro- and reproductive physiology of mollusks and insect cellular defense (Stanley-Samuelson et al., 1991; Stanley-Samuelson, 1994). Eicosanoids have also been detected in a wide diversity of parasite species, including the trematodes *Schistosoma mansoni* and *Fasciola hepatica*, protozoans such as *Trypanosoma brucei* and *Plasmodium falciparum*, the cestode *Spirometra erinaceieuropaei,* and nematodes *Brugia malayi* and *Dirofilaria immitis* (Liu and Weller, 1992; Kubata et al., 1998, 2000; Ali et al., 1999; Noverr et al., 2003; Chaisson and Hallem, 2012). Although the functions of some oxylipins from some parasites are known, considering the diversity of parasite species and the breadth of their host taxa, the functions of oxylipins in host-parasite interactions are largely unexplored (Noverr et al., 2003; Chaisson and Hallem, 2012).

Oxylipins are released by all biota transported within their environment, received and perceived by another individual, leading to possible changes in behavior depending on the receiver's state or species (Baldwin et al., 2006; Fink, 2007). The reception of these chemical cues may trigger chemokinesis and chemiotaxis in a wide diversity of species in response to the presence of a specific cocktail of cues and play an important role in species interactions within aquatic systems (de Petrocellis and di Marzo, 1994; Fink, 2007; Pohnert et al., 2007; Poulson et al., 2009; Brönmark and Hansson, 2012). For example, oxylipin cocktails released from a benthic diatom (*Achnanthes biasolettiana*) were attractive to the Wandering snail (*Radix ovata*) (Fink et al., 2006a). Yet, another diatom species (*Gomphonema parvulum*) was not attractive to the snail, demonstrating that differences in oxylipin cocktails between species were detected and elicited a differential response from the snail (Fink et al., 2006a,b; Fink, 2007).

Preliminary research suggests that oxylipins may strongly influence ecosystem processes and fine-scale community structure, through structuring planktonic food webs, and influencing patchiness of consumers within ecosystems (Pohnert et al., 2007; Moelzner and Fink, 2015a; Saha et al., 2019). Gastropods respond to oxylipin cues from food sources and shift their behavior in response to food resource quality (Moelzner and Fink, 2014, 2015a). As snails actively grazed upon food sources, chemicals released by the snail and its prey provide information about the quality and types of food resources in the environment (Moelzner and Fink, 2015b). The perception of these cues is a potential mechanism behind the patchy distribution of herbivores within communities (Moelzner and Fink, 2015a).

Parasites themselves use chemoperception of host-generated oxylipins to facilitate transmission to hosts (Daugschies and Joachim, 2000; Noverr et al., 2003; Chaisson and Hallem, 2012). For example, host-seeking by sea lice (*Lepeophtheirus salmonis*) is mediated by the parasite's attraction to isophorone, an oxylipin expressed by salmon hosts (*Salmo salar* and *Scophthalmus maximus*) (Ingvarsdóttir et al., 2002; Bailey et al., 2006). Similarly, aquatic transmission of larval blood flukes (*Schistosoma* spp.) to invertebrate (miracidia stage) and vertebrate (cercariae stage) hosts is increased in the presence of particular host-derived oxylipins (Fusco et al., 1986; Allan et al., 2009). In this system, not only do a larger percentage of cercarial blood flukes penetrate hosts, but the parasite also alters the biosynthesis of some of their own oxylipins (Fusco et al., 1986; Nevhutalu et al., 1993; Chaisson and Hallem, 2012). If the host's behavior is affected by this change in the parasite oxylipins, this could be an instance where oxylipins mediate NCEs in host-parasite interactions. But, despite the potential importance of oxylipins in structuring community interactions, oxylipin cocktails are not well studied in aquatic systems and most of this research has focused on two-way interactions between free-living species (Fink, 2007; Saha et al., 2019). Given the preponderance of parasites in aquatic systems, more work is needed to understand the key molecules in chemoperception by parasites, whether parasites alter their oxylipins after receiving host cues, and whether these in changes parasite cues induce changes in community interactions (Fink, 2007; Pohnert et al., 2007; Sures et al., 2017).

PARASITE IMPACTS ON CHEMICAL COMMUNICATION

Parasitism of aquatic hosts can lead to behavioral and physiological changes in the host, including the alteration of feeding rates, stress response, survival, and competition (Park, 1948; Rivero and Ferguson, 2003; Bedhomme et al., 2005; Lefèvre et al., 2009; Friesen et al., 2020). Host behavioral modifications can include changes in activity levels, aggression, boldness, microhabitat use, and attraction of uninfected host species (Kunz and Pung, 2004; Mikheev et al., 2010; Reisinger et al., 2015; Friesen et al., 2018; Eliuk et al., 2020). It is often suggested that chemical cues may be mediating these types of behaviors and interactions albeit through indirect evidence (e.g., Rohr et al., 2009; Eliuk et al., 2020). As a result, our understanding of how parasites may impact chemical communication in an ecological context is not well understood.

Although a few hypotheses have been proposed to explain changes in host behavior, the potential mechanisms, including the role of chemical communication, are rarely explored (Poulin, 2010; Heil, 2016; Herbison et al., 2018). One hypothesis proposes that parasites may cause pathology to their host, and the side effects or byproducts of this pathology may lead to changes in host behavior and physiology (Pohnert, 2002; Poulin, 2010; Heil, 2016). Oxylipins may be one potential mechanism for this hypothesis as oxylipins are released by some hosts in response to an attack, or tissue damage (Morishima et al., 1997; Funk, 2001). Wound-activated lipases can start to liberate oxylipins as disease progresses and even mediate symptoms (Pohnert, 2002; D'Ippolito et al., 2004; Maibam et al., 2014). During this process, the oxylipin cocktail released by the host may include these novel or upregulated oxylipins, altering its composition and thereby affecting interactions relying on this chemical communication.

An alternative hypothesis proposes that an adaptive response to infection may be a mechanism for alterations in host behavior due to parasites (Poulin, 2010). The host may change its behavior or physiology in order to either mitigate the consequences of this infection or to be rid of the infection or infestation all together (Poulin, 2010; Heil, 2016). Oxylipins are known to modulate the immune response of insects and plants in response to pathogens and predation in a variety of ecosystems (Stanley-Samuelson et al., 1991; Noverr et al., 2003; Pozo et al., 2004; Pieterse and Dicke, 2007). Hosts may produce different amounts or combinations of oxylipins in attempts to remove infection (Noverr et al., 2003; Heil, 2016).

Finally, parasites may directly manipulate their hosts through biochemical interference (Poulin, 2010). Many parasite species are known to produce a variety of oxylipins (Noverr et al., 2003; Chaisson and Hallem, 2012). Parasites may use oxylipins to their advantage and produce oxylipins that modulate host immune systems as a way to protect themselves (Noverr et al., 2003). Parasites have evolved mechanisms to suppress the host defense response by interfering with key pathway regulators (Angeli et al., 2001; Pozo et al., 2004). We suggest that altering oxylipins released by the host or the release of oxylipins by the parasite are adaptive avenues by which the parasite could directly modify their host behavior in a manner that would benefit the parasite, such as increased transmission.

Parasite modification of chemical cues released by their host, through any of the mechanisms described above, will affect interactions and other physiological processes involving these cues. Oxylipins have crucial hormone-like functions within species and play roles in secondary metabolite biosynthesis (Holighaus and Rohlfs, 2019). If parasites release novel or higher amounts of oxylipins than their hosts or induce changes in the production of oxylipins by their host, this may induce behavioral changes in their host, such as boldness and feeding rates, as many oxylipins are well-known as extracellular signaling molecules (Noverr et al., 2003; Gabbs et al., 2015). Additionally, if an altered oxylipin cocktail contains novel or an increased amount of oxylipins that induce chemotaxis by conspecifics (e.g., oxylipins used in mate finding, prey seeking), the change in cocktail may lead to attraction of novel hosts (including predators) increasing the chances of successful transmission.

NON-CONSUMPTIVE EFFECTS OF PARASITISM MEDIATED BY ALTERED CUES

To avoid parasitism, host species may adjust their behavior and physiology (Rohr et al., 2009; Horn et al., 2020). These NCEs of parasites are an ecological consequence of novel host responses to infection risk (Rohr et al., 2009; Koprivnikar and Penalva, 2015). The importance of NCEs of parasites and the role of chemical cues in these interactions has been recognized but much is still not understood (Rohr et al., 2009). For example, tadpoles (*Bufo americanus*) exhibited avoidance behavior and elevated activity in response to chemical cues produced by a snail (*Planorbella trivolvis*) shedding *Echinostoma trivolvis* cercariae (Rohr et al., 2009). A potential mechanism for these effects is manipulation of oxylipin cocktails by the parasite, by altering the production of host oxylipins or releasing oxylipins of its own.

If parasites alter the oxylipin cocktail of their host, parasites can alter the signaling web. Other individuals within the community may receive different signals and as a result alter their behavior because of the modifications in the oxylipin cocktails (Vos et al., 2006). In some cases, other conspecifics and heterospecifics may have evolved to respond to these changes, like responses to alarm cues, by reacting to an imminent risk of becoming infected themselves. Alterations could include an uninfected individual actively changing phenotypic traits, like its behavior, physiology, or habitat use to avoid becoming infected (Hill and Weissburg, 2013). A naïve host may prime their immune system in response to the perceived risk of becoming infected, which is likely to be energetically costly (McPherson et al., 2018). The defense priming of other naïve species may have additional consequences to the transmission success of other parasites within the ecosystem, which in turn may have cascading impacts on the entire community (Friesen et al., 2020). As a defense to the perceived risk, individuals may spend time trying to avoid the threat, but in turn this may increase their vulnerability to predation by reducing time spent on effective defenses and foraging, reducing their ability to compete for resources, and reducing energy available to reproduce, develop and/or grow (Koprivnikar and Penalva, 2015). These altered cues within aquatic signaling webs could also provide one mechanism driving the "ecology of fear" in these communities (Buck et al., 2018; Weinstein et al., 2018; Zanette and Clinchy, 2019). Further, modified cues may ultimately lead to altered species abundance and distribution, and competition resulting in potentially dramatic impacts on ecosystems (Thiemann and Wassersug, 2000; Marino et al., 2014; Buck et al., 2018; Horn et al., 2020).

FUTURE DIRECTIONS AND MODEL SYSTEMS

Due to our limited understanding of the extent that oxylipins mediate interactions, including NCEs, many effects of oxylipin modification remain speculative and require further research. Gastropods and trematode parasites are ubiquitous, have essential functional roles, and their interactions are fundamental

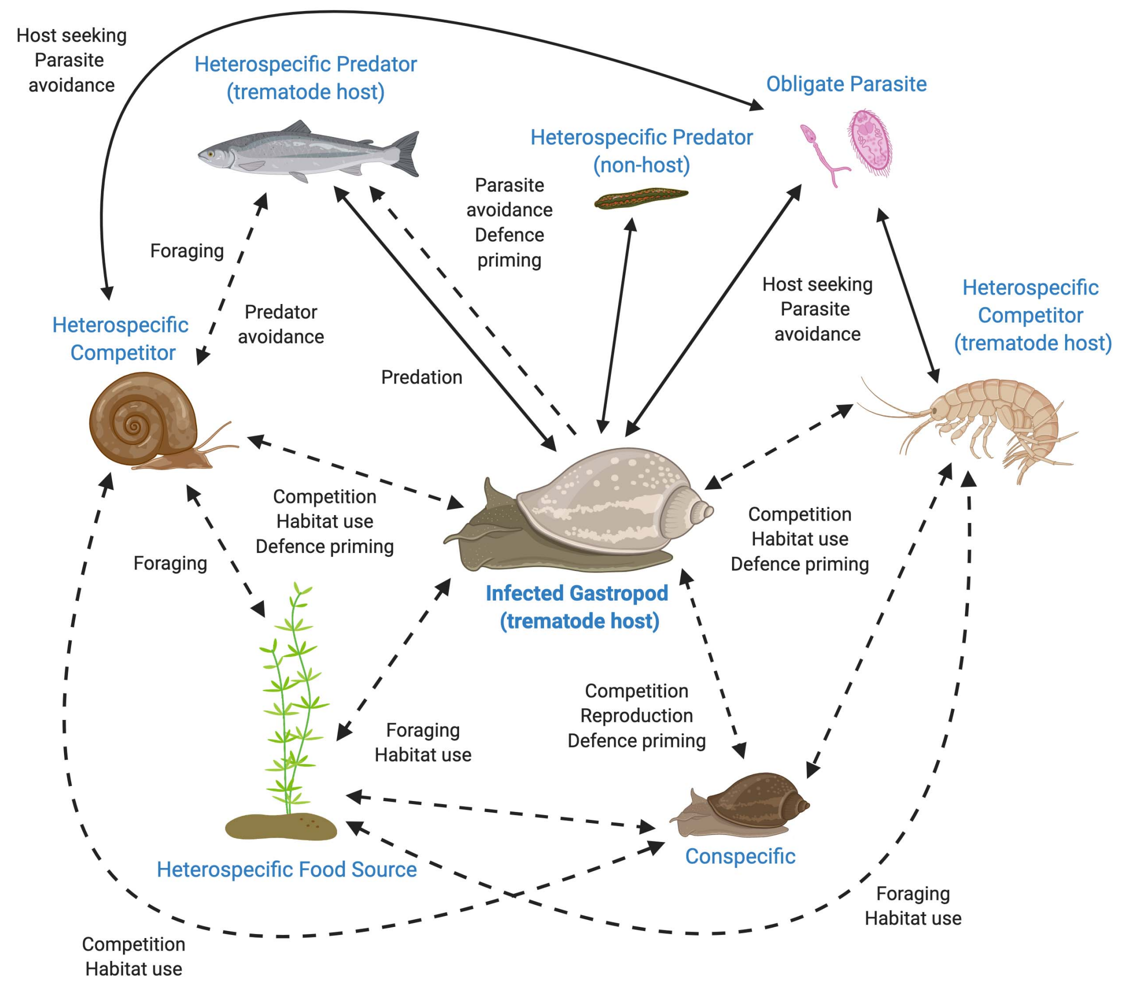

FIGURE 1 | Interactions in a freshwater community potentially mediated by chemical cues originating from a trematode-infected gastropod. Many of these interactions include non-consumptive effects of parasites that may be mediated by oxylipins [Dotted lines (and labels in bold)]. All interactions would be impacted by any change in the gastropod's oxylipins, which would then have far reaching consequences for the surrounding community. Figure created with BioRender.com.

to community dynamics within aquatic ecosystems (Hawkins and Furnish, 1987; Fink et al., 2006a; Wojdak and Mittelbach, 2007; Strong et al., 2008). Thus, they present promising systems to test hypotheses related to the role of oxylipins in mediating NCEs of parasites. Gastropods serve as intermediate hosts to trematode parasites, which infect a wide variety of other host species to complete their life cycles. As trematodes penetrate, migrate, develop, and feed in gastropods, the oxylipin cocktails emitted by these hosts may change. For example, as redial stages of trematodes consume gonad tissue, oxylipins important for reproductive activities of gastropods may be reduced or eliminated. Gastropod hosts may also alter their oxylipin cocktail to increase their reproductive output in order to mitigate the costs of infection, such as a shortened life span or castration (Sorensen and Minchella, 2001).

As gastropods and trematodes are known to both produce and respond to oxylipins and represent a crucial interaction within aquatic communities, these model systems are ideal for testing the role of oxylipins in mediating NCEs of parasites within aquatic environments (see **Figure 1**). Currently, the chemical cues of schistosome and echinostome trematodes have received the most attention (e.g., Haas et al., 1995; Noverr et al., 2003; Chaisson and Hallem, 2012; Langeloh and Seppälä, 2018). These trematodes could be further explored to test if the behavior or physiology (e.g., immune function) of their conspecific or heterospecific snail hosts are altered in response to exposure to the chemical cocktails produced by infected snails compared to cocktails produced by uninfected snails, or trematodes themselves (**Figure 1**; Rohr et al., 2009; Langeloh and Seppälä, 2018; Eliuk et al., 2020). Oxylipin cocktails produced by infected snails can be characterized, isolated and used to directly test the role of chemical signals in mediating NCEs of parasites. Further, exposure to these modified chemical cocktails alone could be used to test differences in behavior by other snails and other aquatic community members, such as their foraging time, habitat use, reproductive output, competition, and the ability to evade predators. The generality and strength of alterations to oxylipins in response to infection and their consequences in mediating interactions could be assessed by testing across the diversity of trematodes and snail hosts, between hosts within a life cycle, and according to host-specificity of the trematode. Further, the dynamic nature of chemical communication in snail trematode-systems can be investigated in different contexts such as in controlled laboratory, semi-natural, and natural conditions.

As oxylipins are impacted by shifts in temperature, we can test whether signaling webs and pathway crosstalk involving gastropods and trematodes are being affected by climate change. Emerging research has demonstrated that alterations in oxylipin cues change the outcome of predator-prey interactions (Zupo et al., 2015). Many non-target individuals will be receiving and responding to parasite-modified cues within the signaling web, which may lead to unpredictable changes to the community (e.g., freshwater fish, Fisher et al., 2006). Given the indirect evidence available, we think it is reasonable to suggest variation in parasite diversity and abundance, which ultimately alters the signaling web of a community, may have similarly dramatic impacts on aquatic communities by mediating NCEs of parasites within the community.

In addition, as trematode parasites are ubiquitous throughout aquatic ecosystems and often dominate the biomass of communities, the alteration of chemical cues, like oxylipin cocktails, through parasite infection, may have far reaching effects, modifying the signaling web and affecting pathway crosstalk (Mitchell, 2003; Kuris et al., 2008; Lagrue and Poulin, 2016; Paseka, 2017). Because gastropod-trematode systems can be readily studied in nature and the lab, the impacts of NCEs of parasites mediated by chemical cues can be explored in a variety of contexts. They provide an ideal opportunity to investigate the role of chemical cues in influencing ecological interactions (from individual to community level) and ecosystem processes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

OF and JD developed and wrote the manuscript. Both authors contributed to the manuscript and approved the submitted version.

FUNDING

Funding for OF was provided by the Weston Family Awards in Northern Research Postdoctoral Fellowship. Funding for JD was through a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant.

ACKNOWLEDGMENTS

We thank Janet Koprivnikar for the invitation (on behalf of the other topic editors) to contribute to this Research Topic. We also thank members of the Detwiler Laboratory for feedback and remarks on a draft of this manuscript, and Jake Stout for initially inspiring our research direction.

REFERENCES

Ali, S. F., Joachim, A., and Daugschies, A. (1999). Eicosanoid production by adult *Fasciola hepatica* and plasma eicosanoid patterns during fasciolosis in sheep. *Int. J. Parasitol.* 29, 743–748. doi: 10.1016/S0020-7519(99)00020-X

Allan, F., Rollinson, D., Smith, J. E., and Dunn, A. M. (2009). Host choice and penetration by *Schistosoma haematobium* miracidia. *J. Helminthol.* 83, 33–38. doi: 10.1017/S0022149X08073628

Angeli, V., Faveeuw, C., Roye, O., Fontaine, J., Teissier, E., Capron, A., et al. (2001). Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal

Langerhans cell migration during schistosomiasis infection. *J. Exp. Med.* 193, 1135–1147. doi: 10.1084/jem.193.10.1135

Bailey, R. J. E., Birkett, M. A., Ingvarsdóttir, A., Mordue, A. J., Mordue, W., O'Shea, B., et al. (2006). The role of semiochemicals in host location and non-host avoidance by salmon louse (*Lepeophtheirus salmonis*) copepodids. *Can. J. Fish. Aquat. Sci.* 63, 448–456. doi: 10.1139/f05-231

Baldwin, I. T., Halitschke, R., Paschold, A., Von Dahl, C. C., and Preston, C. A. (2006). Volatile signaling in plant-plant interactions: "Talking trees" in the genomics era. *Science* 311, 812–815. doi: 10.1126/science.1118446

Bedhomme, S., Agnew, P., Vital, Y., Sidobre, C., and Michalakis, Y. (2005). Prevalence-dependent costs of parasite virulence. *PLoS Biol.* 3:e262. doi: 10.1371/journal.pbio.0030262

Brönmark, C., and Hansson, L.-A. (eds) (2012). *Chemical Ecology in Aquatic Systems*. Oxford: Oxford University Press.

Brönmark, C., and Hansson, L.-A. (2000). Chemical communication in aquatic systems: an introduction. *Oikos* 88, 103–109. doi: 10.1034/j.1600-0706.2000.880112.x

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

Burks, R. L., and Lodge, D. M. (2002). Cued in: advances and opportunities in freshwater chemical ecology. *J. Chem. Ecol.* 28, 1901–1917. doi: 10.1023/A:1020785525081

Caldwell, G. S. (2009). The influence of bioactive oxylipins from marine diatoms on invertebrate reproduction and development. *Mar. Drugs* 7, 367–400. doi: 10.3390/md7030367

Chaisson, K. E., and Hallem, E. A. (2012). Chemosensory behaviours of parasites. *Trends Parasitol.* 28, 427–436. doi: 10.1016/j.pt.2012.07.004.Chemosensory

Daugschies, A., and Joachim, A. (2000). Eicosanoids in parasites and parasitic infections. *Adv. Parasitol.* 46, 181–240. doi: 10.1016/s0065-308x(00)46009-4

de Petrocellis, L., and di Marzo, V. (1994). Aquatic invertebrates open up new perspectives in eicosanoid research: biosynthesis and bioactivity. *Prostaglandins Leukot. Essent. Fat. Acids* 51, 215–229. doi: 10.1016/0952-3278(94)90183-X

D'Ippolito, G., Tucci, S., Cutignano, A., Romano, G., Cimino, G., Miralto, A., et al. (2004). The role of complex lipids in the synthesis of bioactive aldehydes of the marine diatom *Skeletonema costatum*. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1686, 100–107. doi: 10.1016/j.bbalip.2004.09.002

Dicke, M., and Sabelis, M. W. (1988). Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol.* 2, 131–139. doi: 10.2307/2389687

Eliuk, L. K., Brown, S., Wyeth, R. C., and Detwiler, J. T. (2020). Parasite-modified behaviour in non-trophic transmission: trematode parasitism increases the attraction between snail intermediate hosts. *Can. J. Zool.* 98, 417–424. doi: 10.1139/cjz-2019-0251

Ferrari, M. C. O., Wisenden, B. D., and Chivers, D. P. (2010). Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can. J. Zool.* 88, 698–724. doi: 10.1139/Z10-029

Fink, P. (2007). Ecological functions of volatile organic compounds in aquatic systems. *Mar. Freshw. Behav. Physiol.* 40, 155–168. doi: 10.1080/10236240701602218

Fink, P., Von Elert, E., and Jüttner, F. (2006a). Oxylipins from freshwater diatoms act as attractants for a benthic herbivore. *Arch. Hydrobiol.* 167, 561–574. doi: 10.1127/0003-9136/2006/0167-0561

Fink, P., Von Elert, E., and Jüttner, F. (2006b). Volatile foraging kairomones in the littoral zone: attraction of an herbivorous freshwater gastropod to algal odors. *J. Chem. Ecol.* 32, 1867–1881. doi: 10.1007/s10886-006-9115-y

Fisher, H. S., Wong, B. B. M., and Rosenthal, G. G. (2006). Alteration of the chemical environment disrupts communication in a freshwater fish. *Proc. R. Soc. B Biol. Sci.* 273, 1187–1193. doi: 10.1098/rspb.2005.3406

Friesen, O. C., Goellner, S., Poulin, R., and Lagrue, C. (2020). Parasites shape community structure and dynamics in freshwater crustaceans. *Parasitology* 147, 182–193. doi: 10.1017/S0031182019001483

Friesen, O. C., Poulin, R., and Lagrue, C. (2018). Parasite-mediated microhabitat segregation between congeneric hosts. *Biol. Lett.* 14:20170671. doi: 10.1098/rsbl.2017.0671

Funk, C. D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294, 1871–1875. doi: 10.1126/science.294.5548.1871

Fusco, A. C., Salafsky, B., and Delbrook, K. (1986). *Schistosoma mansoni*: production of cercarial eicosanoids as correlates of penetration and transformation. *J. Parasitol.* 72, 397–404. doi: 10.2307/3281679

Gabbs, M., Leng, S., Devassy, J. G., and Aukema, H. M. (2015). Advances in our understanding of oxylipins. *Am. Soc. Nutr.* 6, 513–540. doi: 10.3945/an.114.007732.PUFAs

Haas, W., Haberl, B., Kalbe, M., and Körner, M. (1995). Snail-host-finding by miracidia and cercariae: chemical host cues. *Parasitol. Today* 11, 468–472. doi: 10.1016/0169-4758(95)80066-2

Hawkins, C. P., and Furnish, J. K. (1987). Are snails important competitors in stream ecosystems? *Oikos* 49, 209–220. doi: 10.2307/3566028

Heil, M. (2016). Host manipulation by parasites: cases, patterns, and remaining doubts. *Front. Ecol. Evol.* 4:80. doi: 10.3389/fevo.2016.00080

Herbison, R., Lagrue, C., and Poulin, R. (2018). The missing link in parasite manipulation of host behaviour. *Parasit. Vectors* 11:222. doi: 10.1186/s13071-018-2805-9

Hermann, S. L., and Landis, D. A. (2017). Scaling up our understanding of non-consumptive effects in insect systems. *Curr. Opin. Insect Sci.* 20, 54–60. doi: 10.1016/j.cois.2017.03.010

Hill, J. M., and Weissburg, M. J. (2013). Predator biomass determines the magnitude of non-consumptive effects (NCEs) in both laboratory and field environments. *Oecologia* 172, 79–91. doi: 10.1007/s00442-012-2488-4

Holighaus, G., and Rohlfs, M. (2019). Volatile and non-volatile fungal oxylipins in fungus-invertebrate interactions. *Fungal Ecol.* 38, 28–36. doi: 10.1016/j.funeco.2018.09.005

Horn, C. J., Mierzejewski, M. K., Elahi, M. E., and Luong, L. T. (2020). Extending the ecology of fear: parasite-mediated sexual selection drives host response to parasites. *Physiol. Behav.* 224:113041. doi: 10.1016/j.physbeh.2020.113041

Ingvarsdóttir, A., Birkett, M. A., Duce, I., Genna, R. L., Mordue, W., Pickett, J. A., et al. (2002). Semiochemical strategies for sea louse control: host location cues. *Pest Manag. Sci.* 58, 537–545. doi: 10.1002/ps.510

Kats, L. B., and Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5, 361–394. doi: 10.1080/11956860.1998.11682468

Kaupp, U. B., Hildebrand, E., and Weyand, I. (2006). Sperm chemotaxis in marine invertebrates — molecules and mechanisms. *J. Cell. Physiol.* 208, 487–494. doi: 10.1002/jcp

Kergunteuil, A., Humair, L., Maire, A. L., Moreno-Aguilar, M. F., Godschalx, A., Catalán, P., et al. (2020). Tritrophic interactions follow phylogenetic escalation and climatic adaptation. *Sci. Rep.* 10:2074. doi: 10.1038/s41598-020-59068-2

Kita, M., Kitamura, M., and Uemura, D. (2010). "Pheromones of marine invertebrates and algae," in *Comprehensive Natural Products II Chem. Biol*, Vol. 4, ed. K. Mori (Oxford: Elsevier), 263–281. doi: 10.1016/b978-008045382-8.00097-6

Koprivnikar, J., and Penalva, L. (2015). Lesser of two evils? Foraging choices in response to threats of predation and parasitism. *PLoS One* 10:e0116569. doi: 10.1371/journal.pone.0116569

Kubata, B. K., Duszenko, M., Kabututu, Z., Rawer, M., Szallies, A., Fujimori, K., et al. (2000). Identification of a novel prostaglandin $F_{2\alpha}$ synthase in *Trypanosoma brucei*. *J. Exp. Med.* 192, 1327–1337. doi: 10.1084/jem.192.9.1327

Kubata, B. K., Eguchi, N., Urade, Y., Yamashita, K., Mitamura, T., Tai, K., et al. (1998). *Plasmodium falciparum* produces prostaglandins that are pyrogenic, somnogenic, and immunosuppressive substances in humans. *J. Exp. Med.* 188, 1197–1202. doi: 10.1084/jem.188.6.1197

Kunz, A. K., and Pung, O. J. (2004). Effects of *Microphallus turgidus* (Trematoda: Microphallidae) on the predation, behavior, and swimming stamina of the grass shrimp *Palaemonetes pugio*. *J. Parasitol.* 90, 441–445. doi: 10.1645/GE-183R

Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-Macedo, L., Boch, C. A., et al. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454, 515–518. doi: 10.1038/nature06970

Lagrue, C., and Poulin, R. (2016). The scaling of parasite biomass with host biomass in lake ecosystems: are parasites limited by host resources? *Ecography* 39, 507–514. doi: 10.1111/ecog.01720

Langeloh, L., and Seppälä, O. (2018). Relative importance of chemical attractiveness to parasites for susceptibility to trematode infection. *Ecol. Evol.* 8, 8921–8929. doi: 10.1002/ece3.4386

Lefèvre, T., Lebarbenchon, C., Gauthier-Clerc, M., Missé, D., Poulin, R., and Thomas, F. (2009). The ecological significance of manipulative parasites. *Trends Ecol. Evol.* 24, 41–48. doi: 10.1016/j.tree.2008.08.007

Liu, L. X., and Weller, P. F. (1992). Intravascular filarial parasites inhibit platelet aggregation: role of parasite-derived prostanoids. *J. Clin. Invest.* 89, 1113–1120. doi: 10.1172/JCI115691

Maibam, C., Fink, P., Romano, G., Buia, M. C., Gambi, M. C., Scipione, M. B., et al. (2014). Relevance of wound-activated compounds produced by diatoms as toxins and infochemicals for benthic invertebrates. *Mar. Biol.* 161, 1639–1652. doi: 10.1007/s00227-014-2448-0

Marino, J. A., Holland, M. P., and Middlemis Maher, J. (2014). Predators and trematode parasites jointly affect larval anuran functional traits and corticosterone levels. *Oikos* 123, 451–460. doi: 10.1111/j.1600-0706.2013.00896.x

Martin, L. B., Addison, B. A., Bean, A. G. D., Buchanan, K. L., Crino, O. L., Eastwood, J. R., et al. (2019). Extreme competence: keystone hosts of infections. *Trends Ecol. Evol.* 34, 303–314. doi: 10.1016/j.tree.2018.12.009

McPherson, O. G., Friesen, O. C., Selbach, C., and Poulin, R. (2018). Prior infections or defence priming: what determines the risk of trematode infections in amphipod hosts? *Parasitol. Res.* 117, 1915–1923. doi: 10.1007/s00436-018-5885-8

Metcalf, R. L., and Kogan, M. (1987). Plant volatiles as insect attractants. *Crit. Rev. Plant Sci.* 5, 251–301. doi: 10.1080/07352688709382242

Mikheev, V. N., Pasternak, A. F., Taskinen, J., and Valtonen, E. T. (2010). Parasite-induced aggression and impaired contest ability in a fish host. *Parasit. Vectors* 3:17. doi: 10.1186/1756-3305-3-17

Mitchell, C. E. (2003). Trophic control of grassland production and biomass by pathogens. *Ecol. Lett.* 6, 147–155. doi: 10.1046/j.1461-0248.2003.00408.x

Moelzner, J., and Fink, P. (2014). The smell of good food: volatile infochemicals as resource quality indicators. *J. Anim. Ecol.* 83, 1007–1014. doi: 10.1111/1365-2656.12220

Moelzner, J., and Fink, P. (2015a). Consumer patchiness explained by volatile infochemicals in a freshwater ecosystem. *Ecosphere* 6, 1–15. doi: 10.1890/ES14-00246.1

Moelzner, J., and Fink, P. (2015b). Gastropod grazing on a benthic alga leads to liberation of food-finding infochemicals. *Oikos* 124, 1603–1608. doi: 10.1111/oik.02069

Morishima, I., Yamano, Y., Inoue, K., and Matsuo, N. (1997). Eicosanoids mediate induction of immune genes in the fat body of the silkworm, *Bombyx mori*. *FEBS Lett.* 419, 83–86. doi: 10.1016/S0014-5793(97)01418-X

Nevhutalu, P. A., Salafsky, B., Haas, W., and Conway, T. (1993). Schistosoma mansoni and Trichobilharzia ocellata: comparison of secreted cercarial eicosanoids. *J. Parasitol.* 79, 130–133. doi: 10.2307/3283293

Noverr, M. C., Erb-Downward, J. R., and Huffnagle, G. B. (2003). Production of eicosanoids and other oxylipins by pathogenic eukaryotic microbes. *Clin. Microbiol. Rev.* 16, 517–533. doi: 10.1128/CMR.16.3.517-533.2003

Park, T. (1948). Interspecies competition in populations of *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. *Ecol. Monogr.* 18, 265–307. doi: 10.2307/1948641

Paseka, R. E. (2017). Low parasite biomass in oligotrophic streams differs from previous estimates in aquatic ecosystems. *Freshw. Sci.* 36, 377–386. doi: 10.1086/691471

Pieterse, C. M. J., and Dicke, M. (2007). Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends Plant Sci.* 12, 564–569. doi: 10.1016/j.tplants.2007.09.004

Pohnert, G. (2002). Phospholipase A_2 activity triggers the wound-activated chemical defense in the diatom *Thalassiosira rotula*. *Plant Physiol.* 129, 103–111. doi: 10.1104/pp.010974

Pohnert, G., Steinke, M., and Tollrian, R. (2007). Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. *Trends Ecol. Evol.* 22, 198–204. doi: 10.1016/j.tree.2007.01.005

Poulin, R. (2010). *Parasite Manipulation of Host Behavior: An Update and Frequently Asked Questions*, 1st Edn. Amsterdam: Elsevier Inc. doi: 10.1242/jeb.073353

Poulson, K. L., Sieg, R. D., and Kubanek, J. (2009). Chemical ecology of the marine plankton. *Nat. Prod. Rep.* 26, 729–745. doi: 10.1039/b806214p

Pozo, M. J., Van Loon, L. C., and Pieterse, C. M. J. (2004). Jasmonates - signals in plant-microbe interactions. *J. Plant Growth Regul.* 23, 211–222. doi: 10.1007/s00344-004-0031-5

Reisinger, L. S., Petersen, I., Hing, J. S., Davila, R. L., and Lodge, D. M. (2015). Infection with a trematode parasite differentially alters competitive interactions and antipredator behaviour in native and invasive crayfish. *Freshw. Biol.* 60, 1581–1595. doi: 10.1111/fwb.12590

Rivero, A., and Ferguson, H. M. (2003). The energetic budget of *Anopheles stephensi* infected with *Plasmodium chabaudi*: is energy depletion a mechanism for virulence? *Proc. R. Soc. B Biol. Sci.* 270, 1365–1371. doi: 10.1098/rspb.2003.2389

Rohr, J. R., Swan, A., Raffel, T. R., and Hudson, P. J. (2009). Parasites, info-disruption, and the ecology of fear. *Oecologia* 159, 447–454. doi: 10.1007/s00442-008-1208-6

Saha, M., Berdalet, E., Carotenuto, Y., Fink, P., Harder, T., John, U., et al. (2019). Using chemical language to shape future marine health. *Front. Ecol. Environ.* 17:530–537. doi: 10.1002/fee.2113

Schultz, J. C., and Appel, H. M. (2004). Cross-kingdom cross-talk: hormones shared by plants and their insect herbivores. *Ecology* 85, 70–77. doi: 10.1890/02-0704

Sorensen, R. E., and Minchella, D. J. (2001). Snail-trematode life history interactions: past trends and future directions. *Parasitology* 123, 3–18. doi: 10.1017/s0031182001007843

Stanley, D. W. (2014). "Eicosanoids in invertebrate immunity," in *Eicosanoids in Invertebrate Signal Transduction Systems*, ed. D. W. Stanley (Princeton, NJ: Princeton University Press), 109–148.

Stanley-Samuelson, D. W. (1994). The biological significance of prostaglandines and related eicosanoids in invertebrates. *Am. Zool.* 34, 589–598. doi: 10.1093/icb/34.6.589

Stanley-Samuelson, D. W., Jensen, E., Nickerson, K. W., Tiebel, K., Ogg, C. L., and Howard, R. W. (1991). Insect immune response to bacterial infection is mediated by eicosanoids. *Proc. Natl. Acad. Sci. U.S.A.* 88, 1064–1068. doi: 10.1073/pnas.88.3.1064

Strong, E. E., Gargominy, O., Ponder, W. F., and Bouchet, P. (2008). Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. *Hydrobiologia* 595, 149–166. doi: 10.1007/s10750-007-9012-6

Sures, B., Nachev, M., Pahl, M., Grabner, D., and Selbach, C. (2017). Parasites as drivers of key processes in aquatic ecosystems: facts and future directions. *Exp. Parasitol.* 180, 141–147. doi: 10.1016/j.exppara.2017.03.011

Thiemann, G. W., and Wassersug, R. J. (2000). Patterns and consequences of behavioural responses to predators and parasites in *Rana* tadpoles. *Biol. J. Linn. Soc.* 71, 513–528. doi: 10.1006/bijl.2000.0459

van Poecke, R. M. P., and Dicke, M. (2004). Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. *Plant Biol.* 6, 387–401. doi: 10.1055/s-2004-820887

Vos, M., Vet, L. E. M., Wäckers, F. L., Middelburg, J. J., Van Der Putten, W. H., Mooij, W. M., et al. (2006). Infochemicals structure marine, terrestrial and freshwater food webs: implications for ecological informatics. *Ecol. Inform.* 1, 23–32. doi: 10.1016/j.ecoinf.2005.06.001

Weinstein, S. B., Moura, C. W., Mendez, J. F., and Lafferty, K. D. (2018). Fear of feces? Tradeoffs between disease risk and foraging drive animal activity around raccoon latrines. *Oikos* 127, 927–934. doi: 10.1111/oik.04866

Weissburg, M. J., and Beauvais, J. (2015). The smell of success: the amount of prey consumed by predators determines the strength and range of cascading non-consumptive effects. *PeerJ* 3:e1426. doi: 10.7717/peerj.1426

Wendel, T., and Jüttner, F. (1996). Lipoxygenase-mediated formation of hydrocarbons and unsaturated aldehydes in freshwater diatoms. *Phytochemistry* 41, 1445–1449. doi: 10.1016/0031-9422(95)00828-4

Wojdak, J. M., and Mittelbach, G. G. (2007). Consequences of niche overlap for ecosystem functioning: an experimental test with pond grazers. *Ecology* 88, 2072–2083. doi: 10.1890/06-0651.1

Zanette, L. Y., and Clinchy, M. (2019). Ecology of fear. *Curr. Biol.* 29, R309–R313. doi: 10.1016/j.cub.2019.02.042

Zupo, V., Maibam, C., Buia, M. C., Gambi, M. C., Patti, F. P., Scipione, M. B., et al. (2015). Chemoreception of the seagrass *Posidonia oceanica* by benthic invertebrates is altered by seawater acidification. *J. Chem. Ecol.* 41, 766–779. doi: 10.1007/s10886-015-0610-x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 25 May 2021
doi: 10.3389/fevo.2021.651159

Avoidance of Contaminated Food Correlates With Low Protozoan Infection in Bonobos

Cécile Sarabian[1]*, Raphaël Belais[2] and Andrew J. J. MacIntosh[1]

[1] Primate Research Institute, Kyoto University, Inuyama, Japan, [2] Amis des Bonobos du Congo, Lola ya Bonobo Sanctuary, Kinshasa, Democratic Republic of the Congo

Intense selection pressure from parasites on free-living animals has resulted in behavioral adaptations that help potential hosts avoid sources of infection. In primates, such "behavioral immunity" is expressed in different contexts and may vary according to the ecology of the host, the nature of the infectious agent, and the individual itself. In this study, we investigated whether avoidance of contaminated food was associated with reduced parasite infection in sanctuary-housed bonobos. To do this, we used bonobos' responses to soil- and fecally-contaminated food in behavioral experiments, and then compared the results with an estimate of protozoan infection across individuals. We found that avoidance of contaminated food correlated negatively with *Balantioides coli* infection, a potentially pathogenic protozoan transmitted through the fecal-oral route. The association between avoidance responses and parasitism were most evident in experiments in which subjects were offered a choice of food items falling along a gradient of fecal contamination. In the case of experiments with more limited options and a high degree of contamination, most subjects were averse to the presented food item and this may have mitigated any relationship between feeding decisions and infection. In experiments with low perceived levels of contamination, most subjects consumed previously contaminated food items, which may also have obscured such a relationship. The behavioral immunity observed may be a consequence of the direct effects of parasites (infection), reflecting the first scale of a landscape of disgust: individual responses. Indirect effects of parasites, such as modulation of feeding decisions and reduced social interactions—and their potential trade-offs with physiological immunity—are also discussed in light of individual fitness and primate evolution. This study builds on previous work by showing that avoidance behaviors may be effective in limiting exposure to a wide diversity of oro-fecally transmitted parasites.

Keywords: parasite avoidance, behavioral immunity, *Pan paniscus*, *Balantioides coli*, micro-landscape of disgust

Edited by:
*Sara B. Weinstein,
The University of Utah, United States*

Reviewed by:
*Caroline R. Amoroso,
University of Virginia, United States
Collin James Horn,
University of Alberta, Canada*

***Correspondence:**
*Cécile Sarabian
sarabiancecile@gmail.com*

Specialty section:
This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *08 January 2021*
Accepted: *19 April 2021*
Published: *25 May 2021*

Citation:
Sarabian C, Belais R and MacIntosh AJJ (2021) Avoidance of Contaminated Food Correlates With Low Protozoan Infection in Bonobos. Front. Ecol. Evol. 9:651159. doi: 10.3389/fevo.2021.651159

INTRODUCTION

Intense selection pressure from parasites on free-living animals has driven behavioral adaptations in potential hosts to avoid sources of infection (Curtis, 2014; Sarabian et al., 2018b). The "behavioral immune system" (Schaller and Park, 2011)—also known as the "adaptive system of disgust" (Curtis, 2013; Lieberman and Patrick, 2014)—orchestrates avoidance of parasites through: (1) detection of signals that co-occur with infectious agents; (2) activation of emotional and cognitive responses; and (3) elicitation of behaviors that reduce the risk of disease (Curtis, 2014). Such behavioral immunity arises as a non-consumptive effect (NCE) of parasitism (Buck et al., 2018) and can have significant impacts on an individual, e.g., in its overall patterns of foraging and even in its choice of social partners. In primates, behavioral immunity is evidenced by avoidance of fecally-contaminated food and water (Sarabian and MacIntosh, 2015; Amoroso et al., 2017, 2019; Sarabian et al., 2018a; Poirotte and Kappeler, 2019; Poirotte et al., 2019), reductions in time spent grooming infected conspecifics (Poirotte et al., 2017), avoidance of risky sex with ulcerated mates with a sexually-transmitted disease (Paciência et al., 2019), and recursion to specific sites within a home range according to the intensity of infection within the group and contamination of the environment (Poirotte et al., 2017).

Although studies describing the mechanisms and strategies of parasite avoidance in primates and other animals are increasing, investigation of the consequences of parasite avoidance, its behavioral plasticity and the trade-offs involved remain scarce (Buck et al., 2018; Sarabian et al., 2018b). In non-human primates, to our knowledge, evidence of fitness consequences of behavioral immunity at an individual level is limited at present to two studies showing that hygienic tendencies and personalities (i.e., the persistence of such strategies across time and space) correlate with low levels of geohelminth infection in free-ranging Japanese macaques (*Macaca fuscata*; Sarabian and MacIntosh, 2015) and low oro-fecally transmitted parasite richness in wild gray mouse lemurs (*Microcebus murinus*; Poirotte and Kappeler, 2019). At a social level, a recent framework based on empirical studies illustrates how a change in the network structure, through a decrease in contact rates after a pathogen has been detected, reduces pathogen transmission (Romano et al., 2020). At a higher level of ecological organization, Weinstein et al. (2018) propose a rethink of host behavior through a "landscape of disgust," in which animals would have evolved to navigate through peaks of parasite abundance and valleys of safety—all avoided or reached via different compromises—provoking cascading effects at lower (i.e., individual and social) and higher (ecosystem) levels.

In this article, we focus on the first level of avoidance: individuals and their immediate environment. To do so, we investigated how sanctuary-housed bonobos (*Pan paniscus*) fit into a "micro landscape of disgust," i.e., whether their sensitivity toward surrounding contaminated food is associated with levels of parasite infection. Little is known about the gastrointestinal parasites of these highly social endangered primates in the wild, with only four published studies revealing a similar range of parasite taxa as that described in chimpanzees (*Pan troglodytes*) (Hasegawa et al., 1983; Dupain et al., 2009; Narat et al., 2015; Medkour et al., 2021). Typical protozoa and helminths in *Pan* species include *Troglodytella*, *Balantioides*, *Entamoeba*, *Capillaria*, *Strongyloides*, *Ascaris*, *Trichuris*, *Enterobius*, *Oesophagostomum* spp., and Dicrocoelids (Petrželková and Huffman, 2018). Many of these parasites have a direct life cycle and are transmitted through the fecal-oral route. Through a series of experiments, our previous study revealed a positive correlation between food/surface contamination and bonobo aversion toward potential food items (Sarabian et al., 2018a). Specifically, we observed a lower likelihood of feeding on contaminated food, as well as lower interaction rates and fewer instances of tool use with/through contaminated surfaces. Because we expected to observe variation in oro-fecally/soil transmitted parasite infection in bonobos, we considered results from three of our earlier experiments that focused specifically on the avoidance of feces and soil contamination. These experiments involved food that was either covered with soil/feces, in contact with or near feces, or previously in contact with feces. The logical next step was to investigate the potential correlation between aversion and infection phenotypes in bonobos.

We thus coupled experimental data previously published in Sarabian et al. (2018a) with coproscopic analyses to test the infection-avoidance hypothesis in bonobos. Based on the assumption that the adaptive system of disgust has evolved to procure health benefits (Curtis, 2014), we predicted that higher levels of avoidance would reflect lower levels of parasite infection. Then, given previous reports of variation in behavioral immunity among primates (Moya et al., 2004; Sarabian and MacIntosh, 2015; Sarabian et al., 2018a; Tybur et al., 2018; Paciência et al., 2019; Poirotte and Kappeler, 2019), we also predicted that the patterns of infection would vary according to the sex and age of each individual—with males and immatures being more prone to infection (i.e., higher parasite infection intensity and/or richness) than females and mature individuals, respectively.

MATERIALS AND METHODS

Study Site and Bonobos

The experiments were conducted between May and July 2016 with semi-free-ranging bonobos at Lola ya Bonobo Sanctuary[1] in Kinshasa, Democratic Republic of the Congo (for further details see Sarabian et al., 2018a). During the day, bonobos live in four separate enclosures including high canopy forest areas with palm oil trees, swamps or water ponds (E1–E3), and an outdoor forested playground ("nursery"). At night, they are socially housed in dormitories composed of different compartments with several hammocks. To facilitate maintenance, each outdoor enclosure and dormitory has doors with a grid of metal bars through which bonobos can pass an arm and be examined by caretakers and veterinarians. In addition to the fruits, leaves and palm oil nuts (*Elaeis guineensis*) that naturally occur in their enclosures, bonobos were fed twice daily with 6.5 kg of seasonal fruits and vegetables, as well as sugarcane, soy milk, boiled eggs and peanuts.

Behavioral Experiments

In this study, we considered three experiments for which experimental results were previously published in Sarabian et al. (2018a), and which involved feeding decisions on soil- and/or fecally-contaminated food under different contamination contexts (i.e., high, gradient, past). We classified the bonobos into two age groups for both males and females: immature

---

[1] www.lolayabonobo.org

(infants and juveniles; 2–7 years) and mature (adolescents and adults; 8–22 years; see **Supplementary Table 1** for details). To match with fecal sampling data (below), we considered a total of 39 individuals with 34 (22 matures; 17 females) in experiment 1, 33 (21 matures; 15 females) in experiment 2, and 32 (19 matures; 14 females) in experiment 3 (**Supplementary Table 1**). The first experiment aimed to test for bonobos' avoidance of highly contaminated food by presenting three slices of apple—one of their favorite food items at the sanctuary (Rosati and Hare, 2011, 2013)—simultaneously: one slice covered with soil, one slice covered with feces, and one slice clean (control). The second experiment explored bonobos' sensitivity to a gradient of contamination risk by presenting a "chain of contagion"; a single row of six banana slices with one of the slices at the extremity placed atop feces from a conspecific (**Figure 1A**). The third experiment examined whether bonobos are sensitive to previous contamination (with conspecific feces), potentially reflecting a lower perceived level of contamination. The experimenter presented two slices of banana, one put in contact with feces, the other not. The feces were then removed from view using a cardboard cover and the two slices of banana were presented to the subject. For each of these experiments, items were presented on a table behind enclosures' doors from where we recorded each subject's feeding decision.

In exp. 1, we considered the feeding response to contaminated apple slices (1 if they fed on soil- and/or feces-covered slices—see Results for details; 0 if they only fed on control items). In exp. 2, since our previous study (Sarabian et al., 2018a) reported feeding on contaminated (slices #1–2; atop and adjacent to feces, respectively) and uncontaminated slices of banana (#3–6), as well as not feeding at all despite approaching the experimental area, we considered three "feeding" categories ("contaminated"; "uncontaminated"; and "none") instead of a binary response. The category "none" reflects a subject who approached to within 1 m of the experimental set-up—sometimes engaging with it (by e.g., touching the table/a food item or using a stick to reach the table)—but did not feed on any slice of banana. In exp. 3, we considered the feeding response to previously contaminated bananas (1 if they fed on the contaminated piece; 0 if they fed on the control only). Subjects were all tested in the morning/afternoon before feeding occurred to minimize the effect of potential satiety. All

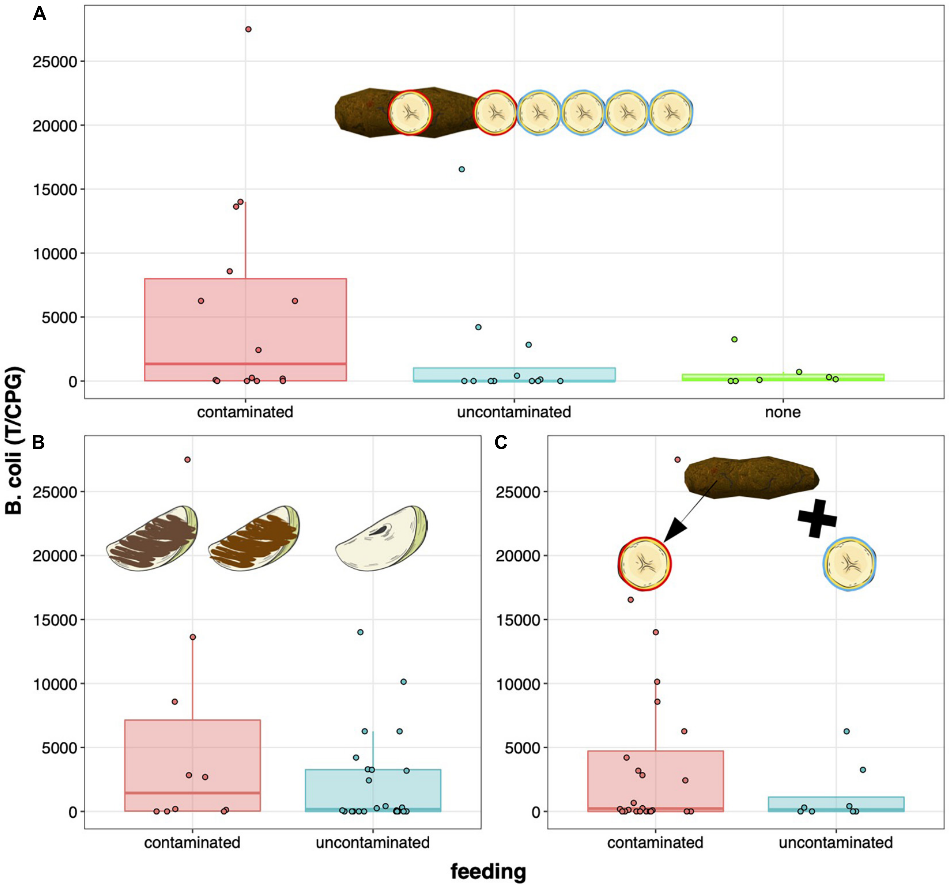

FIGURE 1 | Levels of *Balantioides coli* infection (average number of trophozoites/cysts per gram of feces) according to feeding decisions in bonobos exposed to **(A)** a chain of contagion in exp. #2; **(B)** soil- and fecally-contaminated apple slices in exp. #1; and **(C)** previous contamination in exp. #3. Individuals (dots) feeding on contaminated food (red boxplots) tended to be significantly more infected with *B. coli* than individuals feeding on control food (blue boxplots) or not feeding at all (green boxplot in exp. #2).

experiments and measured variables are described in greater detail in Sarabian et al. (2018a).

Fecal Sample Collection and Parasitological Analyses

Fecal samples were collected over the second half of June 2016 after bonobos awoke from sleep. The last anthelmintic treatment (Albendazole 400 mg) took place at the beginning of March 2016 (Mungongo et al., unpublished data) and no antiprotozoal treatments were conducted in the months preceding our study. Approximately 2 g of fecal matter was put in a 15 mL tube filled with sodium acetate-acetic acid-formaldehyde (SAF) solution. We processed 93 samples from 39 identified individuals (2.4 ± 1.3 samples individual^{-1}; **Supplementary Table 1**) using fecal sedimentation and flotation (for a subset only; see below) procedures to identify and quantify parasite stages in feces (see Pomajbíková and Huzová, 2018). We used fecal egg/trophozoite/cyst counts (EPG: number of eggs gram^{-1} fecal sediment for metazoan parasites; T/CPG: number of trophozoites/cysts gram^{-1} fecal sediment for protozoan parasites) determined via microscopy using a McMaster slide as a proxy for parasite infection across subjects. The fecal sediment of each sample was screened 3 times (3 single McMaster chamber replicates; 3 chambers in total) and the average was used to calculate values of EPG or T/CPG. To test whether these counts were reliable, a second observer screened 11% ($N = 10$) of the samples using the same method and count procedures. In addition, the second observer processed the same 10 samples through different flotation solutions (Sheather's sugar, Zinc sulfate, Sodium nitrate). Floated samples were screened twice (2 double McMaster chamber replicates; 4 chambers in total) and the average EPG or T/CPG was computed. We used Spearman's rank correlation tests to compare for each parasite species: (1) inter-observer reliability examined after fecal sedimentation; and (2) inter-method reliability with the same observer. To maximize analytical efficiency in relating behavior with infection, we retained only parasites that reached a threshold of 30% prevalence in our samples. If results from our reliability tests across concentration methods differed, we used only the most sensitive method in our analyses.

Statistical Analyses

We built generalized linear mixed-effects models (GLMMs) to analyze how infection levels may covary with feeding decisions across experiments. Three models were constructed—one for each experiment—with the average EPG or T/CPG (across counts and samples) of each individual used as a count response. Each model used a negative binomial error structure to reflect the distribution of the EPG or T/CPG data. For predictor variables, we included feeding decision (#1: feed on contaminated apple slices or not; #2: feed on contaminated/uncontaminated banana slices or not; #3: feed on previously contaminated banana or not), and a combined age-sex category (mature female, mature male, immature female, immature male). Because we did not aim to explicitly test the mean difference between bonobos in different enclosures, we placed enclosure identity (E1, E2, E3, nursery) as a random effect in the models. Moreover, our study did not involve multiple trials per individual in the same experiment, so a repeated design was not necessary. All three models were fit using the package *glmmTMB* (Magnusson et al., 2020). We used the package *lmtest* (Hothorn et al., 2020) to compare full (fitted) models with all predictor variables included vs. null models with only variables to control for, i.e., "age-sex category" as a fixed effect and "enclosure" as a random effect, using likelihood ratio tests (LRT). When possible, we also compared full (fitted) models with potential interactions between terms of interest (i.e., feeding decisions and age-sex category; Sarabian et al., 2018a) vs. full models without interactions using LRT. Finally, we used the *DHARMa* package (Hartig, 2021) to test for homogeneity of residuals, homoscedasticity across variables, and the absence of zero-inflation in our data and no violations of model assumptions were found. All data were analyzed in R v.3.6.3 (R Core Team, 2020) and are accessible in the **Supplementary Material**.

RESULTS

Which Parasites Infect Bonobos at Lola ya Bonobo?

The most common parasite observed in our subjects with a prevalence of 64% was *Balantioides coli* (formerly known as *Balantidium coli*; Pomajbíková and Modrý, 2018); a protozoan acquired through the ingestion of fecally-contaminated food and/or water. The prevalence of the other protozoan found in the samples, *Troglodytella* sp., was 23%. Apart from these two protozoans, we did not detect any other gastrointestinal parasites during our examinations. Moreover, out of the 14 fecal samples in which *T.* sp. was detected, *B. coli* was detected only twice. T/CPG of the observed organisms derived from fecal sedimentation were highly correlated across observers (Spearman's rank correlation; *Balantioides coli* obs1 vs. obs2: $r = 0.92$; $p < 0.001$; *Troglodytella* sp. CPG obs1 vs. obs2: $r = 1$; $p < 0.001$; see **Supplementary Material**). However, a correlation matrix of CPG counts for the 10 samples analyzed across all methods (sedimentation vs. flotation) did not always show high reliability for *B. coli* (Spearman's rank correlation; sedimentation vs. sugar: $r = 0.41$; $p = 0.073$; vs. zinc sulfate: $r = 0.66$; $p = 0.002$; vs. sodium nitrate: $r = 0.38$; $p = 0.096$). Correlations were better for the detection of *Troglodytella* sp. (Spearman's rank correlation; sedimentation vs. sugar: $r = 0.86$; $p < 0.001$; vs. zinc sulfate: $r = 0.86$; $p < 0.001$; vs. sodium nitrate: $r = 0.74$; $p < 0.001$). Fecal sedimentation provided the highest sensitivity for diagnostics of *B. coli*, and the largest counts, so we used these data to calculate T/CPG.

Does Avoidance of Contaminated Food Relate to Infection?

The proportions of individuals feeding on contaminated food in experiments #1–3 were 0.29, 0.42 and 0.75, respectively. Note that only 2 out of 34 individuals fed on fecally-contaminated apple slices in exp. 1, hence the regrouping of soil- and fecally-contaminated apple slices as "contaminated food" (**Figure 1B**). Only model #2 significantly outperformed its respective null model (LRT; #1: ΔLogLik = 0.37, Δd.f. = 1, $p = 0.389$; #2:

ΔLogLik = 3.67, Δd.f. = 2, $p = 0.026$; #3: ΔLogLik = 0.79, Δd.f. = 1, $p = 0.208$; see **Supplementary Table 2** for details). We thus retained the full model (#2) without interaction between the age-sex category and feeding because the latter was not outperformed by the model with interaction (LRT; ΔLogLik = 5.17, Δd.f. = 6, $p = 0.112$). We found that *B. coli* infection (T/CPG) was significantly lower in subjects who fed on uncontaminated banana slices and in others who did not feed compared to those who fed on contaminated slices in exp. 2 (GLM#2; uncontaminated vs. contaminated: $z = -2.73$, $p = 0.006$; none vs. contaminated: $z = -3.13$, $p = 0.002$; **Figure 1A** and **Table 1**). Moreover, subjects who did not feed and those who fed on uncontaminated banana slices did not significantly differ in levels of *B. coli* infection ($z = -0.10$, $p = 0.923$; **Table 1**). We also found that mature males were infected to a higher degree with *B. coli* than other age-sex categories (all $0.006 < p \leq 0.07$; see **Table 1** for details)—a pattern we did not find in null models #1 and #3.

DISCUSSION

This study revealed a negative correlation between bonobos' aversion to contaminated food and infection with an oro-fecally transmitted and potentially pathogenic gastrointestinal parasite (*Balantioides coli*). We previously showed that bonobo feeding decisions were influenced by contamination risk, with individuals avoiding food associated with sensory cues of soil and/or conspecific feces (Sarabian et al., 2018a). Here, we show that bonobos who are sensitive to gradients of risk (exp. 2) may benefit from reduced parasite burdens. As was shown in previous studies of Japanese macaques (Sarabian and MacIntosh, 2015) and gray mouse lemurs (Poirotte and Kappeler, 2019), our results indicate that risk-averse bonobos may therefore have a fitness advantage over risk-prone individuals in their ability to minimize oro-fecally transmitted parasite acquisition. If so, our study supports the infection-avoidance hypothesis in a new host-parasite system, and one with close phylogenetic ties to our own human lineage.

Yet, the detection of a significant correlation between aversion and infection could only be highlighted in a certain context. In exp. 1, the level of contamination was quite high for two-thirds of the food items (slices of apple) presented—with about the same weight of contaminant (soil or feces) as the slice. Consequently, 71% of subjects only fed on control (clean) apples. This "high contamination—high aversion" context may not have allowed enough behavioral variation to reflect a difference in the magnitude of infection among "avoiders" and "risk takers" (**Figure 1B**). In comparison, contamination in exp. 2 followed a gradient as one slice of banana (on 6) was deposited atop feces, a second next to it and others in contact with the previous one. In this condition, 58% of subjects restricted themselves to uncontaminated slices (#3–6)

TABLE 1 | Factors affecting variation in levels of *Balantioides coli* infection (CPG).

Statistical model	Predictor variable	est.	s.e.	stat.	P
[1] Variation in *B. coli* infection (exp.1)	(Intercept)	7.944	0.884	8.987	<2e−16***
	Age.sex (imm.f vs. mat.m)	0.496	1.494	0.332	0.740
	Age.sex (imm.m vs. mat.m)	−0.127	1.489	−0.085	0.932
	Age.sex (mat.f vs. mat.m)	0.151	1.252	0.121	0.904
	Age.sex (imm.f vs. mat.f)	0.342	1.492	0.229	0.819
	Age.sex (imm.m vs. mat.f)	−0.271	1.492	−0.181	0.856
	Age.sex (imm.f vs. imm.m)	0.610	1.697	0.360	0.719
[2] Variation in *B. coli* infection (exp. 2)	(Intercept)	12.38	1.694	7.311	3e−13***
	Feeding (none vs. contaminated)	−4.657	1.488	−3.130	**1.8e−3****
	Feeding (uncontaminated vs. contaminated)	−4.502	1.648	−2.733	**6.3e−3****
	Feeding (none vs. uncontaminated)	−0.164	1.689	−0.097	0.923
	Age.sex (imm.f vs. mat.m)	−3.774	2.083	−1.812	0.070
	Age.sex (imm.m vs. mat.m)	−4.178	1.539	−2.715	**6.6e−3****
	Age.sex (mat.f vs. mat.m)	−3.953	1.711	−2.310	**0.021***
	Age.sex (imm.f vs. mat.f)	0.179	1.671	0.107	0.915
	Age.sex (imm.m vs. mat.f)	−0.229	1.551	−0.147	0.883
	Age.sex (imm.f vs. imm.m)	0.405	1.735	0.233	0.815
[3] Variation in *B. coli* infection (exp. 3)	(Intercept)	8.332	0.923	9.022	<2e−16***
	Age.sex (imm.f vs. mat.m)	0.101	1.556	0.065	0.949
	Age.sex (imm.m vs. mat.m)	−0.665	1.483	−0.449	0.654
	Age.sex (mat.f vs. mat.m)	−0.558	1.423	−0.392	0.695
	Age.sex (imm.f vs. mat.f)	−1.947	2.100	−0.927	0.354
	Age.sex (imm.m vs. mat.f)	−2.514	1.870	−1.344	0.179
	Age.sex (imm.f vs. imm.m)	0.767	1.703	0.450	0.653

*Bold text denotes predictor variables with significant variation in the response. Significant effects are marked: ***(p < 0.001), **(p < 0.01), *(p < 0.05). Statistical models are labeled with a number in square parentheses that reflects the experiment number referred to in the text. Note that for models [1] and [3], only the control variables are presented as the full models did not outperform their respective nulls.*

or did not feed at all. Comparatively, contamination in exp. 3 only refers to a previous contamination event: one slice of banana was put in contact with conspecific feces for 5 s (see Sarabian et al., 2018a) before being presented to the tested subject along the control slice. Thus, only 25% of subjects restricted their feeding to the control item, which may reflect lower perceived levels of contamination (**Figure 1C**). The validity of the model and significance of the negative correlation between feeding decisions and infection in exp. 2 may be explained by the gradient of avoidance that could be expressed along that gradient of contamination. Indeed, *B. coli* T/CPG were not only significantly different between feeders of contaminated banana slices and non-feeders, but also between feeders of contaminated slices and feeders of non-contaminated slices (**Figure 1A**). However, non-feeders and feeders of non-contaminated slices did not significantly differ in their levels of infection. These results support the hypothesis that caution (e.g., opting for non-contaminated food or refraining altogether from feeding in potentially risky contexts) reduces infection risk, while also highlighting potential NCEs related to such feeding trade-offs.

Intestinal protozoa such as *Balantioides coli* (primarily known for colonizing the intestine of suids) are common parasites in non-human primates and can also infect humans (Petrželková and Huffman, 2018; Pomajbíková and Modrý, 2018; Poirotte and Charpentier, 2020). In African great apes, though, *B. coli* is more commonly found in captive groups than in their wild counterparts (Pomajbíková et al., 2010). One hypothesis for this difference may be the higher exposure to synanthropic rats in captivity, which have been pointed out as potential reservoirs of infection (Bogdanovich, 1955; Knezevich, 1998). Previous studies have also found an association between a starch-rich diet in captive chimpanzees and crop-raiding baboons (*Papio anubis*) and high intensities of *B. coli* infection (Schovancová et al., 2013; Weyher et al., 2006). Bonobos at Lola ya Bonobo did not rely especially on starchy food—although tubers, bananas and peanuts made up to 30% of their diet at the time of the study. Although *Balantioides* infections are usually asymptomatic in captive great apes, they can cause balantidiasis, with symptoms ranging from mild diarrhea to fulminating dysentery (see Pomajbíková and Modrý, 2018). Some individuals did have high infection intensities, with the most heavily infected individual being a 4-years-old female, born at the sanctuary and living in enclosure 3 (**Supplementary Table 1**). In comparison, *Troglodytella* sp. had a much lower prevalence (23%), which would not allow statistical modeling. Moreover, *T.* sp. was negatively associated with *B. coli*—a trait also observed in chimpanzees, potentially because the latter taxon makes the large intestine less suitable for *T. abrassarti* (McLennan et al., 2017). Most importantly, while *B. coli* is transmitted via the fecal-oral route and considered to be mildly pathogenic in chimpanzees (Pomajbíková et al., 2010), *T.* sp. is not, and is rather viewed as a symbiont with the function of participating in food digestion (Profousová et al., 2011).

Regardless of its pathogenicity, one important aspect of *B. coli* regarding our experiments is its transmission pathway. Infections are usually acquired by ingesting cysts in contaminated food or water (Schuster and Ramirez-Avila, 2008), or otherwise from contaminated substrata. Thus, if the parasite is less prevalent in bonobos that are more averse to contaminated foods, as our study found, this would suggest that avoidance is effective in reducing transmission via the fecal-oral route. These results build on previous findings in other primate species demonstrating a negative correlation between avoidance behaviors and infection with oro-fecally transmitted parasites (Sarabian and MacIntosh, 2015; Poirotte and Kappeler, 2019). To our knowledge, this is the first study to display a link between feces avoidance and the intensity of *B. coli* infection. Our previous work with Japanese macaques focused on intensity of infection with nematode parasites (Sarabian and MacIntosh, 2015), and Poirotte and Kappeler (2019) used parasite status/richness (i.e., number of nematode species present) as a proxy of infection in gray mouse lemurs. Thus, our results add to the growing body of literature showing that sensitivity to contamination can drive feeding decisions in primates, as they do in other taxa (Ezenwa, 2004; Anderson and McMullan, 2018; Coulson et al., 2018; Zélé et al., 2019).

Also in line with previous studies of primates, and according to our prediction, we also observed a significant male bias toward infection. Mature males exhibited significantly higher infection intensities than mature females and immature males. Recent analyses of fecal samples from wild bonobos also found a higher prevalence of gastrointestinal helminths in males compared to females, although no significant differences in estimated infection intensities between the sexes (Dardel, 2020). On the whole, therefore, results in bonobos appear in line with general patterns of male-biased parasitism in mammals (Zuk and McKean, 1996; Klein, 2000, 2004). From a behavioral perspective—in our experiments, at least—this is not intuitive as immatures fed on contaminated food and interacted with contaminated surfaces more often than mature bonobos, including males, and we observed little difference in the risk sensitivity of adult males and females (Sarabian et al., 2018a). Thus, while we cannot rule out behavior as a mechanism underlying male biases in infection among bonobos, other mechanisms may be at play. In wild male chimpanzees, testosterone and cortisol are associated with higher helminth and protozoa richness (Muehlenbein, 2006), which may be explained by the immunosuppressive activity of both hormones (reviewed in Sapolsky et al., 2000; Muehlenbein and Bribiescas, 2005). In turn, levels of fecal testosterone vary with the dominance rank of the individual, so that higher ranking individuals express higher testosterone levels and greater helminth (but not protozoan) richness (Muehlenbein and Watts, 2010). Further studies should thus investigate whether there is a link between dominance rank and levels of gastrointestinal parasite infection in bonobos, and whether their behavioral immunity has a role to play in this equation.

Related to an individual's sex and age, its physiological resistance to or tolerance of infection may also have played a role in shaping patterns of infection observed here. For protozoa such as *B. coli*—which can fully replicate inside their hosts and cause chronic infections

(Ponce-Gordo and García-Rodríguez, 2020)—the physiological immune response is particularly relevant in minimizing the load during or after establishment of the parasite. Hosts can handle parasitism in three ways, known as: avoidance, resistance and tolerance (Rivas et al., 2014). A previous study in salmonids (*Salmo salar* and *Salmo trutta*) showed the potential interplay between these defensive strategies, with i.e., the most resistant populations being the less avoidant and tolerant toward eye fluke (*Diplostomum pseudospathaceum*) infection (Klemme et al., 2020). We acknowledge that focusing on one mechanism could have ignored how other defense strategies may impact the "avoidance-infection" relationship. There is evidence supporting the hypothesis that behavioral and physiological responses are correlated across species (Hawley et al., 2011). For example, promiscuous primates appear to invest more in the production of immune cells such as white blood cells (Nunn et al., 2000; Nunn, 2002), while social insects express reduced immunity to parasites compared to asocial insects (e.g., Barribeau et al., 2015; López-Uribe et al., 2016). Both examples suggest an interplay between behavior and immune physiology. Whether such interactions also exist within species like bonobos, expressed through variable behavioral and immune phenotypes across individuals, has not yet been tested and would be a great start to have a more comprehensive view of parasite defense strategies in these social and endangered primates.

In conclusion, the behavioral immunity observed in bonobos may be a consequence of the direct effects of parasites (infection), reflecting on the first scale of a landscape of disgust: individual responses. However, indirect effects of parasites (i.e., NCEs) such as modulation of feeding decisions and social interactions also exist, and are often accompanied by trade-offs. As such, Japanese macaques would avoid feeding atop conspecific feces for a grain of wheat, but not for half a peanut (Sarabian and MacIntosh, 2015). Similarly, mandrills (*Mandrillus sphinx*) would avoid grooming group members highly infected with oro-fecally transmitted protozoa, except if they are maternal kin (Poirotte and Charpentier, 2020). Although the parasites in question here (i.e., oro-fecally transmitted helminth and protozoa) are not highly pathogenic, the fact that their main contaminant (i.e., feces) can harbor a wide diversity of pathogens, may have led primates to evolve a general avoidance response toward unknown fecal material (see Poirotte et al., 2019). The latter would provide increased fitness in "hygienic" individuals and may have precluded the need to develop cognitively demanding and costly detection and discrimination mechanisms specifically toward helminths and protozoa. Such a strategy may reflect innate tendencies to avoid feces, but could also be acquired through associative learning, which would explain why immatures are less cautious around risky contaminants. Mechanisms for such conditioning may include the association of feces sensory cues with digestive discomfort due to parasite infections acquired from contact with feces (Amoroso, 2021), and/or observational learning from adults making their own foraging decisions (e.g., Huffman and Hirata, 2004; Tarnaud and Yamagiwa, 2008). In sum, we highlight that the micro-landscape of disgust in a social primate can be dynamic and affected by multiple factors, behavior being among them. We thus encourage future studies to explore other correlates of the micro-landscape of disgust in animals.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Welfare and Animal Care Committee of Kyoto University Primate Research Institute (#2016-138).

AUTHOR CONTRIBUTIONS

CS and AM designed the study and wrote the manuscript. CS collected and analyzed the data. RB supported data collection at Lola ya Bonobo Sanctuary. All authors approved the final manuscript.

FUNDING

CS was supported by the Leading Graduate Program in Primatology and Wildlife Science of Kyoto University, the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and is currently funded by the Japan Society for the Promotion of Science (#P19088). This study was supported in part by the Wild and Wise Collaborative Learning Program ("Short-term Intern Program in Primatology and Wildlife Science") of the Kyoto University Primate Research Institute (KUPRI).

ACKNOWLEDGMENTS

We thank Fanny Minesi, Pierrot Mbonzo, Claudine André, and all the staff at Lola ya Bonobo for facilitating data collection; the Ministry of Research and the Ministry of Environment in the Democratic Republic of the Congo for providing permits; and Ashutosh Khandual for performing part of the parasitological analyses. We also thank two reviewers and Marie Sigaud for commenting on an earlier draft of this manuscript, which considerably improved it, as well as Prof. Val Curtis, who inspired the "chain of contagion" experiments and much more in this field of research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.651159/full#supplementary-material

REFERENCES

Amoroso, C. R. (2021). Integrating concepts of physiological and behavioral resistance to parasites. *Front. Ecol. Evol.* 9:635607. doi: 10.3389/fevo.2021.635607

Amoroso, C. R., Frink, A. G., and Nunn, C. L. (2017). Water as a counterstrategy to faecally transmitted disease: an experimental study in captive lemurs. *Behaviour* 154, 1239–1258. doi: 10.1163/1568539X-00003466

Amoroso, C. R., Kappeler, P. M., Fichtel, C., and Nunn, C. L. (2019). Fecal contamination, parasite risk, and waterhole use by wild animals in a dry deciduous forest. *Behav. Ecol. Sociobiol.* 73:153. doi: 10.1007/s00265-019-2769-6

Anderson, A., and McMullan, R. (2018). Neuronal and non-neuronal signals regulate *Caenorhabditis elegans* avoidance of contaminated food. *Phil. Trans. R. Soc. B* 373: 20170255. doi: 10.1098/rstb.2017.0255

Barribeau, S. M., Sadd, B. M., du Plessis, L., Brown, M. J. F., Buechel, S. D., Cappelle, K., et al. (2015). A depauperate immune repertoire precedes evolution of sociality in bees. *Gen. Biol.* 16:83. doi: 10.1186/s13059-015-0628-y

Bogdanovich, V. V. (1955). Spontaneous balantidiosis in rats. *Meditsinskaya Parazitologiya I Parazitarnye Bolezni* 24, 326–329.

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

Coulson, G., Cripps, J. K., Garnick, S., Bristow, V., and Beveridge, I. (2018). Parasite insight: assessing fitness costs, infection risks and foraging benefits relating to gastrointestinal nematodes in wild mammalian herbivores. *Phil. Trans. R. Soc. B* 373:20170197. doi: 10.1098/rstb.2017.0197

Curtis, V. A. (2013). *Don't Look, Don't Touch: The Science Behind Revulsion*. Oxford: Oxford University Press.

Curtis, V. A. (2014). Infection-avoidance behaviour in humans and other animals. *Trends Immunol.* 35, 457–464. doi: 10.1016/j.it.2014.08.006

Dardel, R. (2020). *Suivi Longitudinal des Helminthes Intestinaux D'une Communauté de Bonobos (Pan paniscus)* en Mosaïque Forêt-Savane. Toulouse: Université Paul-Sabatier de Toulouse, 71–81. DVM thesis.

Dupain, J., Nell, C., Petrželková, K. J., Garcia, P., Modrý, D., and Gordo, F. P. (2009). "Gastrointestinal parasites of bonobos in the Lomako Forest, Democratic Republic of Congo," in *Primate Parasite Ecology: The Dynamics of Host-Parasite Relationships*, eds M. A. Huffman, and C. A. Chapman, (Cambridge: Cambridge University Press), 297–310.

Ezenwa, V. (2004). Selective defecation and selective foraging: antiparasite behavior in wild ungulates? *Ethology* 110, 851–862. doi: 10.1111/j.1439-0310.2004.01013.x

Hartig, F. (2021). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. R Package Version 0.4.0*. Available online at: https://cran.r-project.org/web/packages/DHARMa/index.html (accessed April 8, 2021).

Hasegawa, H., Kano, T., and Mulawa, M. (1983). A parasitological survey on the feces of pygmy chimpanzees, *Pan paniscus*, at Wamba, Zaire. *Primates* 24, 419–423.

Hawley, D., Etienne, R. S., Ezenwa, V. O., and Jolles, A. E. (2011). Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Int. Comp. Biol.* 51, 528–539. doi: 10.1093/icb/icr062

Hothorn, T., Zeileis, A., Farebrother, R. W., Cummins, C., Millo, G., and Mitchell, D. (2020). *Lmtest: Testing Linear Regression Models*. R Package Version 0.9-38.

Huffman, M., and Hirata, S. (2004). An experimental study of leaf swallowing in captive chimpanzees: insights into the origin of a self-medicative behavior and the role of social learning. *Primates* 45, 113–118. doi: 10.1007/s10329-003-0065-5

Klein, S. L. (2000). The effects of hormones on sex differences in infection: from genes to behavior. *Neurosci. Biobehav. Rev.* 24, 627–638. doi: 10.1016/S0149-7634(00)00027-0

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunol.* 26, 247–264. doi: 10.1111/j.0141-9838.2004.00710.x

Klemme, I., Hyvärinen, P., and Karvonen, A. (2020). Negative associations between parasite avoidance, resistance and tolerance predict host health in salmonid fish populations. *Proc. R. Soc. B* 287:20200388. doi: 10.1098/rspb.2020.0388

Knezevich, M. (1998). Geophagy as a therapeutic mediator of endoparasitism in a free-ranging group of Rhesus Macaques (*Macaca mulatta*). *Am. J. Primatol.* 44, 71–82. doi: 10.1002/(SICI)1098-2345199844:1<71::AID-AJP6<3.0.CO;2-U

Lieberman, D., and Patrick, C. (2014). Are the behavioral immune system and pathogen disgust identical? *Evol. Behav. Sci.* 8, 244–250. doi: 10.1037/ebs0000018

López-Uribe, M. M., Sconiers, W. B., Frank, S. D., Dunn, R. R., and Tarpy, D. R. (2016). Reduced cellular immune response in social insect lineages. *Biol. Lett.* 12:20150984. doi: 10.1098/rsbl.2015.0984

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., et al. (2020). *Package 'glmmTMB'*. R package version 1.0.2.1.

McLennan, M. R., Hasegawa, H., Bardi, M., and Huffman, M. A. (2017). Gastrointestinal parasite infections and self-medication in wild chimpanzees surviving in degraded forest fragments within an agricultural landscape mosaic in Uganda. *PLoS One* 12:e0180431. doi: 10.1371/journal.pone.0180431

Medkour, H., Castaneda, S., Amona, I., Fenollar, F., André, C., Belais, R., et al. (2021). Potential zoonotic pathogens hosted by endangered bonobos. *Sci. Rep.* 11:6331. doi: 10.1038/s41598-021-85849-4

Moya, J., Bearer, C. F., and Etzel, R. A. (2004). Children's behavior and physiology and how it affects exposure to environmental contaminants. *Pediatrics* 113, 996–1006.

Muehlenbein, M. P. (2006). Intestinal parasite infections and fecal steroid levels in wild chimpanzees. *Am. J. Phys. Anthropol.* 130, 546–550. doi: 10.1002/ajpa.20391

Muehlenbein, M. P., and Bribiescas, R. G. (2005). Testosterone-mediated immune functions and male life histories. *Am. J. Hum. Biol.* 17, 527–558. doi: 10.1002/ajhb.20419

Muehlenbein, M. P., and Watts, D. P. (2010). The costs of dominance: testosterone, cortisol, and intestinal parasites in wild male chimpanzees. *BioPsychoSoc. Med.* 4:21. doi: 10.1186/1751-0759-4-21

Narat, V., Guillot, J., Pennec, F., Lafosse, S., Grüner, A. C., Simmen, B., et al. (2015). Intestinal helminths of wild bonobos in forest-savanna mosaic: risk assessment of cross-species transmission with local people in the Democratic Republic of the Congo. *EcoHealth* 12, 621–633. doi: 10.1007/s10393-015-1058-8

Nunn, C. L. (2002). A comparative study of leukocyte counts and disease risk in primates. *Evolution* 56, 177–190. doi: 10.1111/j.0014-3820.2002.tb00859.x

Nunn, C. L., Gittleman, J. L., and Antonovics, J. (2000). Promiscuity and the primate immune system. *Science* 290, 1168–1170. doi: 10.1126/science.290.5494.1168

Paciência, F. M. D., Rushmore, J., Chuma, I. S., Lipende, I. F., Caillaud, D., Knauf, S., et al. (2019). Mating avoidance in female olive baboons (*Papio anubis*) infected by *Treponema pallidum*. *Sci. Adv.* 5:eaaw9724. doi: 10.1126/sciadv.aaw9724

Petrželková, K. J., and Huffman, M. A. (2018). "Parasites of chimpanzees and bonobos," in *Parasites of Apes: An Atlas of Coproscopic Diagnostics*, eds D. Modrý, B. Pafèo, K. J. Petrželková, and H. Hasegawa, (Frankfurt: Edition Chimaira), 56–63.

Poirotte, C., and Charpentier, M. (2020). Unconditional care from close maternal kin in the face of parasites. *Biol. Lett.* 16:20190869. doi: 10.1098/rsbl.2019.0869

Poirotte, C., and Kappeler, P. (2019). Hygienic personalities in wild grey mouse lemurs vary adaptively with sex. *Proc. R. Soc. B* 286:20190863. doi: 10.1098/rspb.2019.0863

Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., et al. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Sci. Adv.* 3:e1601721. doi: 10.1126/sciadv.1601721

Poirotte, C., Sarabian, C., Ngoubangoye, B., MacIntosh, A. J. J., and Charpentier, M. (2019). Faecal avoidance differs between the sexes but not with nematode infection risk in mandrills. *Anim. Behav.* 149, 97–106. doi: 10.1016/j.anbehav.2019.01.013

Pomajbíková, K., and Huzová, Z. (2018). "Coproscopic examination techniques," in *Parasites of Apes: An Atlas of Coproscopic Diagnostics*, eds D. Modrý, B. Pafčo, K. J. Petrželková, and H. Hasegawa, (Frankfurt: Edition Chimaira), 112–115.

Pomajbíková, K., and Modrý, D. (2018). "*Balantioides coli* and related cyst-forming ciliates," in *Parasites of Apes: An Atlas of Coproscopic Diagnostics*, eds D. Modrı,

B. Pafčo, K. J. Petrželková, and H. Hasegawa, (Frankfurt: Edition Chimaira), 112–115.

Pomajbíková, K., Petrželková, K. J., Profousová, I., and Modrý, D. (2010). Discrepancies in the occurrence of *Balantidium coli* between wild and captive African great apes. *J. Parasitol.* 96, 1139–1144. doi: 10.1645/GE-2433.1

Ponce-Gordo, F., and García-Rodríguez, J. J. (2020). Balantioides coli. *Res. Vet. Sci.* 135, 424–431. doi: 10.1016/j.rvsc.2020.10.028

Profousová, I., Mihaliková, K., Laho, T., Váradyová, Z., Petrželková, K. J., Modrý, D., et al. (2011). The ciliate, *Troglodytella abrassarti*, contributes to polysaccharide hydrolytic activities in the chimpanzee colon. *Folia Microbiol.* 56, 339–343. doi: 10.1007/s12223-011-0053-x

R Core Team, (2020). *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rivas, F. V., Chervonsky, A. V., and Medzhitov, R. (2014). ART and immunology. *Trends Imm.* 35:451. doi: 10.1016/j.it.2014.09.002

Romano, V., MacIntosh, A. J. J., and Sueur, C. (2020). Stemming the flow: information, infection and social evolution. *Trends Ecol. Evol.* 35, 849–853. doi: 10.1016/j.tree.2020.07.004

Rosati, A. G., and Hare, B. (2011). Chimpanzees and bonobos distinguish between risk and ambiguity. *Biol. Lett.* 7, 15–18. doi: 10.1098/rsbl.2010.0927

Rosati, A. G., and Hare, B. (2013). Chimpanzees and bonobos exhibit emotional responses to decision outcomes. *PLoS One* 8:e63058. doi: 10.1371/journal.pone.0063058

Sapolsky, R. M., Romero, M., and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endoc. Rev.* 21, 55–89. doi: 10.1210/edrv.21.1.0389

Sarabian, C., Curtis, V., and McMullan, R. (2018b). Evolution of pathogen and parasite avoidance behaviours. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170256. doi: 10.1098/rstb.2017.0256

Sarabian, C., Belais, R., and Maclntosh, A. J. J. (2018a). Feeding decisions under contamination risk in bonobos. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170195. doi: 10.1098/rstb.2017.0195

Sarabian, C., and MacIntosh, A. J. J. (2015). Hygienic tendencies correlate with low geohelminth infection in free-ranging macaques. *Biol. Lett.* 11:20150757. doi: 10.1098/rsbl.2015.0757

Schaller, M., and Park, J. H. (2011). The behavioral immune system (and why it matters). *Curr. Dir. Psych. Sci.* 20, 99–103. doi: 10.1177/0963721411402596

Schovancová, K., Pomajbíková, K., Procházka, P., Modrý, D., Bolechová, P., and Petrželková, K. J. (2013). Preliminary insights into the impact of dietary starch on the ciliate, *Neobalantidium coli,* in captive chimpanzees. *PLoS One* 8:e81374. doi: 10.1371/journal.pone.0081374

Schuster, F. L., and Ramirez-Avila, L. (2008). Current world status of Balantidium coli. *Clin. Microbiol. Rev.* 21, 626–638. doi: 10.1128/CMR.00021-08

Tarnaud, L., and Yamagiwa, J. (2008). Age-dependent patterns of intensive observation on elders by free-ranging juvenile Japanese macaques (*Macaca fuscata yakui*) within foraging context on Yakushima. *Am. J. Primatol.* 70, 1103–1113. doi: 10.1002/ajp.20603

Tybur, J. M., C1nar, C., Karinen, A. K., and Perone, P. (2018). Why do people vary in disgust? *Phil. Trans. R. Soc. B* 373:20170204. doi: 10.1098/rstb.2017.0204

Weinstein, S. B., Buck, J. C., and Young, H. S. (2018). A landscape of disgust. *Science* 359, 1213–1214. doi: 10.1126/science.aas8694

Weyher, A. H., Ross, C., and Semple, S. (2006). Gastrointestinal parasites in crop raiding and wild foraging *Papio anubis* in Nigeria. *Int. J. Primatol.* 27, 1519–1534. doi: 10.1007/s10764-006-9089-1

Zélé, F., Santos-Matos, G., Figueiredo, A. R. T., Eira, C., Pinto, C., Laurentino, T. G., et al. (2019). Spider mites escape bacterial infection by avoiding contaminated food. *Oecologia* 189, 111–122. doi: 10.1007/s00442-018-4316-y

Zuk, M., and McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. *Int. J. Parasitol.* 26, 1009–1023. doi: 10.1016/S0020-7519(96)80001-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

REVIEW
published: 14 June 2021
doi: 10.3389/fevo.2021.667030

Risk-Induced Trait Responses and Non-consumptive Effects in Plants and Animals in Response to Their Invertebrate Herbivore and Parasite Natural Enemies

Janet Koprivnikar[1]*†, *Alicia Rochette*[2]† *and Mark R. Forbes*[2]†

[1] *Department of Chemistry and Biology, Ryerson University, Toronto, ON, Canada,* [2] *Department of Biology, Carleton University, Ottawa, ON, Canada*

Edited by:
Justin Wright,
Duke University, United States

Reviewed by:
Evan Preisser,
University of Rhode Island,
United States
Michael Sheriff,
University of Massachusetts
Dartmouth, United States

***Correspondence:**
Janet Koprivnikar
jkoprivn@ryerson.ca

†*These authors have contributed equally to this work*

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *11 February 2021*
Accepted: *19 May 2021*
Published: *14 June 2021*

Citation:
Koprivnikar J, Rochette A and Forbes MR (2021) Risk-Induced Trait Responses and Non-consumptive Effects in Plants and Animals in Response to Their Invertebrate Herbivore and Parasite Natural Enemies. Front. Ecol. Evol. 9:667030. doi: 10.3389/fevo.2021.667030

Predators kill and consume prey, but also scare living prey. Fitness of prey can be reduced by direct killing and consumption, but also by non-consumptive effects (NCEs) if prey show costly risk-induced trait responses (RITRs) to predators, which are meant to reduce predation risk. Recently, similarities between predators and parasites as natural enemies have been recognized, including their potential to cause victim RITRs and NCEs. However, plant-herbivore and animal host-parasite associations might be more comparable as victim-enemy systems in this context than either is to prey-predator systems. This is because plant herbivores and animal parasites are often invertebrate species that are typically smaller than their victims, generally cause lower lethality, and allow for further defensive responses by victims after consumption begins. Invertebrate herbivores can cause diverse RITRs in plants through various means, and animals also exhibit assorted RITRs to increased parasitism risk. This synthesis aims to broadly compare these two enemy-victim systems by highlighting the ways in which plants and animals perceive threat and respond with a range of induced victim trait responses that can provide pre-emptive defense against invertebrate enemies. We also review evidence that RITRs are costly in terms of reducing victim fitness or abundance, demonstrating how work with one victim-enemy system can inform the other with respect to the frequency and magnitude of RITRs and possible NCEs. We particularly highlight gaps in our knowledge about plant and animal host responses to their invertebrate enemies that may guide directions for future research. Comparing how potential plant and animal victims respond pre-emptively to the threat of consumption *via* RITRs will help to advance our understanding of natural enemy ecology and may have utility for pest and disease control.

Keywords: herbivore, parasite, induced, non-consumptive effect, defense, natural enemy, risk

INTRODUCTION

On the surface, a tomato plant reacting to the presence of a hungry hornworm caterpillar and a squirrel to that of ticks in the vicinity might seem to have little in common. However, both may respond to the potential risk posed by these different natural enemies prior to any actual attack in surprisingly similar ways, and with broad similarities to that of animal prey responses to predators. Importantly, potential prey can respond to predation risk through various trait changes, from increased physiological stress to altered behaviors (see reviews by Peacor et al., 2013; Sheriff and Thaler, 2014; Sheriff et al., 2020b). For instance, prey will often avoid foraging in areas, or at times, with high predation risk (reviewed by Lima and Dill, 1990), and predator exposure can elevate levels of hormones in prey that are associated with a stress response (e.g., Dahl et al., 2012). Some of these induced prey responses may be costly (e.g., Sheriff et al., 2009), but broadly serve to reduce the odds of predator encounter, as well as better resist attack (reviews by Peacor et al., 2020; Sheriff et al., 2020b; Wirsing et al., 2021).

Critically, enemy **risk-induced trait responses** (RITRs) could result in **non-consumptive effects** (NCEs; see **Glossary** for list of commonly-used terms) if there are fitness costs to potential victims, or reductions in their abundance, irrespective of an actual attack involving consumption by the enemy (Peacor et al., 2020; Sheriff et al., 2020b). For example, female snowshoe hares exposed to non-contact simulated predation had smaller litters, and offspring in poorer condition, than unexposed hares (Sheriff et al., 2009). Such work subsumed under the "ecology of fear" has highlighted that predator NCEs should be considered alongside **consumptive effects** (CEs) in terms of implications for prey population dynamics, with a need for more empirical work to understand better their relative contributions and context-dependency (Sheriff et al., 2020b; Wirsing et al., 2021). In addition, enemy RITRs can affect how potential victims interact with other species, resulting in **trait-mediated indirect effects** (TMIEs) with community-level consequences (reviewed for prey-predator systems by Werner and Peacor, 2003). Prey-predator systems have been the primary focus for NCEs and TMIEs, but there are growing efforts to synthesize this area by considering other natural enemies, such as parasites and pathogens (e.g., Raffel et al., 2008; Buck et al., 2018; Weinstein et al., 2018a; Daversa et al., 2021).

The threat of infection by parasites or pathogens (hereafter, parasites) can cause various responses by potential animal hosts, including many behaviors that serve to avoid parasite contact or reduce the chances of parasite establishment (see reviews by Hart, 1990, 2011). Some of these RITRs may have associated costs that lead to NCEs. For instance, small mammals and larval amphibians will forego foraging opportunities in areas containing helminth infectious stages or ectoparasites (Fritzsche and Allan, 2012; Koprivnikar and Penalva, 2015). As with predator RITRs, those exhibited by potential hosts to reduce their risk of parasitism could also result in TMIEs (Buck and Ripple, 2017).

While it is important and useful to consider and contrast animal prey-predator and host-parasite interactions in the context of natural enemy ecology, certain characteristics of these two victim-enemy systems are different. Predators can be larger than their victims, and consumption is typically immediate, short-term, and lethal for prey; in comparison, hosts are typically larger than their parasites, and consumption by the latter is often not lethal (Lafferty and Kuris, 2002; Raffel et al., 2008; Cortez and Duffy, 2020). For these reasons and others detailed below, we suggest that plants and their invertebrate herbivores represent a victim-enemy system which may provide a particularly useful comparison to that of animal host-parasite. It is our hope that this comparison will help us better understand the varied scope and mechanistic underpinnings of enemy RITRs, including their capacity to affect victim fitness and abundance (via NCEs), or influence other community members (via TMIEs).

In this synthesis, we highlight commonalities and differences between plant-herbivore and animal host-parasite systems that could help direct future studies of RITRs and NCEs for both victim-enemy types, as well as assist in broadly integrating knowledge of natural enemy ecology. We consider RITRs as a temporal sequence of key steps that can lead to NCEs (see **Figure 1**), comparing known and possible aspects for both animal and plant victims of invertebrate parasites and herbivores, respectively. We first examine victim perception of cues related to enemy risk (pre- and post-contact, but without consumption), followed by trait responses in potential victims, and then whether such RITRs can lead to NCEs (population-level consequences), or TMIEs (community-level implications). We do not seek to comprehensively review plant and animal defensive responses to their natural enemies, or to detail the entire suite of RITRs, NCEs and TMIEs reported to date; rather, we aim to highlight how work with one victim-enemy system can potentially inform the other.

NATURAL ENEMY SYSTEMS: PLANT-HERBIVORE VS. ANIMAL HOST-PARASITE ATTRIBUTES

To the best of our knowledge, a paradigm involving plants and animals as victims of invertebrate herbivores and parasites in the context of RITRs and NCEs has neither been posited nor evaluated. As victims, there are certain key biological and ecological similarities in how plants and animals interact with their invertebrate herbivore and parasite enemies, respectively—more so than either of these compared to prey and predators, even though comparisons between the latter and host-parasite associations have been the primary focus thus far (e.g., Raffel et al., 2008; Buck and Ripple, 2017; Daversa et al., 2021). Considering similarities between plants and animals as victims of predators has been useful for evaluating the timing of defensive investment and fitness loss (Sheriff et al., 2020a). Comparing these two taxa as victims of parasites has also provided a framework for better understanding ecological immunity and infection tolerance (Baucom and de Roode, 2011). Additionally, considering strategies for parasite avoidance by animals may inform research into the ways in which plants "avoid" their parasites (Buck et al., 2018). Could comparisons of plant-herbivore and animal-parasite systems also be informative?

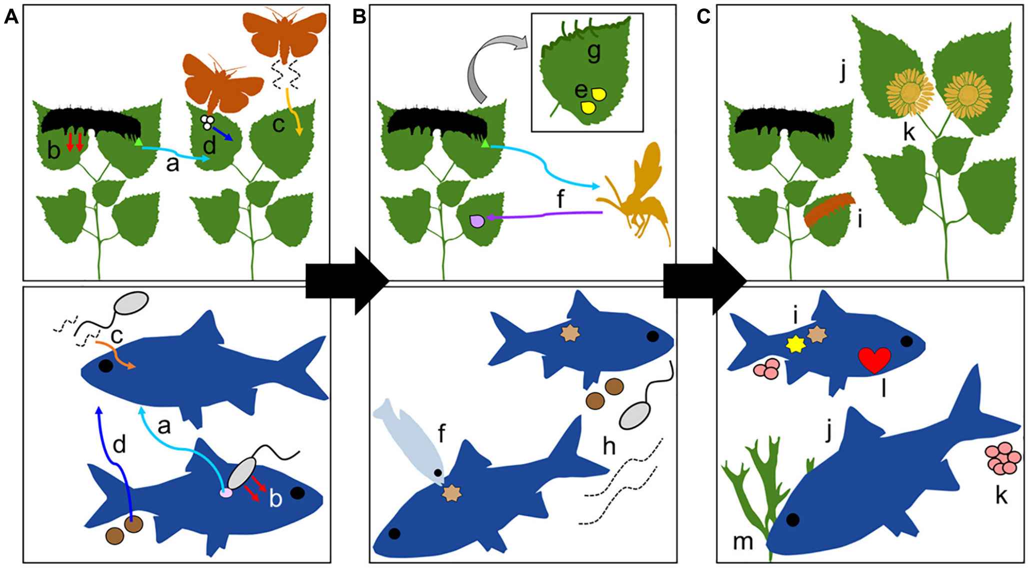

FIGURE 1 | Select broad similarities between plant-herbivore and host-parasite systems for the temporal sequence of events that can cause these natural enemies to have non-consumptive effects (NCEs): **(A)** perception of respective natural enemies; **(B)** enemy risk-induced trait responses (RITRs) related to victim resistance; and, **(C)** potential NCEs of enemy on victim. Specific pathways that are shared or unique between plant and animal victims for **(A–C)**: (a) chemical(s) generated by conspecific under attack, (b) tactile enemy perception, (c) enemy-generated chemical(s), i.e., kairomones, (d) risk proxies (conspecific feces, herbivore eggs), (e) production of secondary metabolites, (f) solicitation of enemy removal (chemical signals, energetic rewards), (g) morphology (trichomes, toughness), (h) spatial avoidance (conspecifics, enemies, proxies), (i) susceptibility to multiple enemies, (j) reduced growth or condition, (k) reduced reproduction, (l) metabolic costs, (m) altered foraging. Original silhouettes obtained from PhyloPic (http://phylopic.org).

Whereas plant and animal victims of herbivores and parasites, respectively, have certain inherent differences (e.g., plants cannot flee herbivores), these victims also share biological similarities relevant for enemy risk perception and costly trait responses. Notably, invertebrate herbivores and animal parasites from a wide range of taxa with differing ecologies pose a ubiquitous threat to their potential victims through their sheer diversity and abundance (Howe and Jander, 2008; Buck et al., 2018). While we focus on herbivorous arthropods given the substantial and long-standing threat that they pose to plants (Howe and Jander, 2008), we also consider other invertebrates (e.g., mollusks). As such, "herbivore(s)" hereafter refers to invertebrate consumers of plant material. Importantly, because parasites of animals are also overwhelmingly invertebrates (Leung, 2014), they are more directly comparable to invertebrate herbivores as enemies.

As noted above, plants and animals not only both face constant challenges from a diverse array of invertebrate enemies, but their relationships with the latter also share important similarities. First, plant and animal victims tend to be larger in size relative to their herbivores and parasites, which is only sometimes the case for animal predators and their prey. Secondly, given the sheer number of species represented by invertebrate herbivores and animal parasites (Howe and Jander, 2008), many have a high degree of specificity for particular victims (Bernays and Graham, 1988; Combes, 2001). Last, and most important, consumption by herbivores or parasites of their respective victims is rarely lethal, in contrast to predators of animal prey (Buck and Ripple, 2017; Cortez and Duffy, 2020; Sheriff et al., 2020a). While enemy encounter is likely relatively frequent and prolonged, the nature and capacity for victim damage by herbivores and parasites is limited compared to damage to animal prey caused by predators.

Notably, how parasites or herbivores attack and damage their victims typically differs from predator-prey systems in terms of modularity—only one specific part of the victim may be targeted, resulting in relatively localized harm. Parasites typically confine themselves to specific host tissues or organs (Adamson and Caira, 1994), and the compartmentalized nature of plants means that an attack on one part (e.g., a single leaf) may be analogous to that on a single individual (Karban et al., 2016). Wound-induced vascular leakage is thus less problematic for plants and hosts compared to prey. Predators also frequently consume so much of their victim such that surviving the attack is not possible—unlike other natural enemies. As such, a host under parasite attack likely faces a threat of further consumption, just as do plants attacked by herbivores. In contrast, future risk of predation should not be an issue when prey is being consumed by a predator.

For these reasons, invertebrate herbivores may be more like parasites than predators (Raffel et al., 2008). We recognize that the distinction is somewhat fluid—e.g., attack from parasitoids might be more similar to attack from predators than from parasites or herbivores. In the case of highly lethal enemies such as parasitoids and predators, we might thus expect particularly strong pre-emptive defenses in potential victims because post-attack responses are limited. Conversely, there is potential for further defensive responses after encounter is initiated for both plant-herbivore and animal-parasite associations—this potential, if realized, might place a constraint on the evolution of flexible, pre-emptive defenses in those two victim-enemy systems. When considering the evolution of pre-emptive defenses in the form of RITRs, and the NCEs associated with them, it is therefore important to bear in mind any contexts in which pre-emptive defenses might not be expressed.

In terms of defense, plants can invest in **tolerance** and/or **resistance** against herbivores (Agrawal and Fishbein, 2008; Kant et al., 2015), as can animal hosts against parasites (Sheldon and Verhulst, 1996; Råberg et al., 2009). In stark contrast, although some prey are capable of escape through autotomy or other strategies, it is not intuitive how most animal prey would invest in tolerance against a predator attack. As such, plant-herbivore and animal host-parasite systems could be particularly useful to compare in the context of enemy RITRs related to resistance. Notably, the **allocation costs** associated with phenotypic plasticity in traits related to victim resistance are central to evaluating NCEs (Peacor et al., 2020). Because both of these victim types may face trade-offs between investments in resistance and tolerance, this could constrain enemy risk-induced pre-emptive defenses compared to those of animal prey in response to predators. We thus expect pre-emptive defenses to be more common, and perhaps stronger, for prey than for plants and animal hosts—the latter two victim types have alternative avenues for adaptive responses available to them. In addition, victims that are likely to incur large costs if attacked, such as fitness losses, are predicted to perceive and respond to elevated enemy risk relatively early in the attack sequence compared to organisms with lower proportional cost(s) if attacked (Sheriff et al., 2020a). Plant and animal hosts are more similar in the expectation of delayed timing of a response than either is to prey.

The following subsections thus consider a temporal sequence involving perception of enemy risk, defensive trait changes in response to the risk posed (RITRs), and the putative costs (NCEs) of those responses for plant-herbivore and animal host-parasite systems (**Figure 1**). We also briefly consider the potential for TMIEs in both victim-enemy systems.

Perception of Enemy Risk

Plants and animals clearly have different sensory structures and capabilities, yet both may perceive cues related to the risk of attack by herbivores or parasites, respectively, in similar ways—just as for their perception of predators (Sheriff et al., 2020a). These natural enemies may be perceived pre-contact, during physical contact before consumption occurs, or even after some minor attack that has not yet incurred meaningful energetic costs to the victim. Because herbivores and parasites take some time to establish a successful feeding (consumptive) relationship compared to predators of animals (Raffel et al., 2008), this post-contact (but pre-consumption) time lag may represent a source of NCEs that is fairly unique to these two types of natural enemy, but also affects victim reliance on pre- vs. post-contact risk cues. Importantly, if consumption does not almost immediately begin after enemy contact, then potential victims of parasites or herbivores could still have time to react as compared to animal prey (Rigby et al., 2002), and thus rely relatively less on pre-contact signals of increased risk.

Pre-contact, plants and animals may perceive cues emanating from the enemy itself, e.g., chemicals such as kairomones (Ruther et al., 2002), or from conspecifics that have made contact or are undergoing consumption (Hart, 1990; Kant et al., 2015; Behringer et al., 2018). While the latter can clearly result in direct fitness reductions (CEs) for the individual(s) being consumed (and their kin), there is enormous potential to generate cues that contain information about the threat posed by shared enemies, thereby causing enemy RITRs which can cause NCEs in other individuals not under attack. Actual physical contact is also perceived by potential victims through various means, and can trigger responses to avoid progression into the consumption phase. Lacking eyes and ears, plants primarily rely on chemical and tactile cues related to herbivore presence.

There are a variety of ways by which plants can directly perceive their herbivorous natural enemies (see reviews by Howe and Jander, 2008; Fürstenberg-Hägg et al., 2013; Aljbory and Chen, 2018). Some of these cues do not require physical contact between the plant and herbivore, while others come into play after initial contact has been made, but before consumption begins (or in its initial stages before a plant response curtails the attack). Pre-contact, plants can perceive herbivore-generated chemicals used for intraspecific communication, meaning that pheromones can also act as kairomones if they have interspecific effects (Ruther et al., 2002). For instance, cotton plants exhibited a defensive response to boll weevil aggregation pheromones (Magalhães et al., 2019).

Post-contact, chemical and tactile cues appear to play roles in directly detecting risks from herbivory, such as snail mucous trails that induced a defensive response in thale cress (Falk et al., 2014). Other important post-contact chemical signals include arthropod oral secretions and excrement (frass), as well as plant cell wall fragments (Howe and Jander, 2008; Fürstenberg-Hägg et al., 2013; Ray et al., 2015, 2016; Aljbory and Chen, 2018). A heightened risk of herbivory can be sensed by tactile cues alone. Plants can detect various vibrations emanating from herbivores, such as those generated by chewing (e.g., Kollasch et al., 2020). Even light touches by arthropod feet can be perceived *via* plant epidermal hairs (trichomes) and elicit defensive responses (e.g., Peiffer et al., 2009). Not surprisingly, oviposition by herbivorous arthropods induces defense-related trait responses, following from both chemical (such as adherents) and tactile elements (reviewed by Hilker and Meiners, 2006).

Plants can gather information about the threat posed by herbivores through indirect means as well. Chemical cues from other individuals undergoing attack may play a particularly important role in enemy RITRs. Notably, plants rapidly emit biogenic volatile organic compounds (VOCs) in response to the first signs of consumption by herbivores (reviews by Howe and Jander, 2008; Aljbory and Chen, 2018; Ameye et al., 2018). Of herbivore-induced plant volatiles (HIPVs), green leaf volatiles (GLVs), which are comprised of six-carbon compounds (e.g., alcohols, aldehydes and esters), represent a particularly important subgroup. HIPVs in this subgroup can act as repellents in herbivore defense, but also induce or prime plant resistance against herbivory (Ameye et al., 2018).

The primary function of such HIPVs appears to be the rapid communication of risk to other parts of the same individual not yet under attack; internal chemical signals are less effective given the modular structure of most plants, thus airborne volatiles convey information faster (Fürstenberg-Hägg et al., 2013). In addition, plant parts that are in similar spatial locations may not share much vascular connection (e.g., two leaves from separate main-stem branches can be next to each other spatially but far apart vascularly). Recognition of these airborne chemicals in turn triggers molecular and physiological cascades that can induce trait changes related to resistance. Intriguingly, there is evidence that other plants (of the same or different species) may "eavesdrop" on such chemical signals, exhibiting trait changes in response to perceived enemy risk (reviews by Karban et al., 2014; Ameye et al., 2018; Bouwmeester et al., 2019). For example, undamaged, neighboring lima bean plants were able to recognize HIPVs from attacked plants, activating a defensive response in the form of extra-floral nectaries to attract enemies of herbivores (Kost and Heil, 2006).

In a similar vein, chemicals are thought to play a large role in animal perception of parasite risk considering that hosts are invariably larger than parasite infective stages (Lafferty and Kuris, 2002), and the latter are typically limited in their motility and generation of audiovisual cues. Whereas plants do not have eyes and ears to detect herbivores, such sensory structures, while present in animals, may be of limited use for gauging risk from minute infective stages of parasites. Chemical and tactile cues are thus expected to be more important signals of parasite presence than audiovisual cues. Like plants, potential animal victims respond to pre-contact chemical signals directly generated by the natural enemy, as well as those from conspecifics being attacked or consumed (Curtis, 2014; Buck et al., 2018).

In terms of chemical cues, animals may use parasite-associated proxies to gauge infection risk (Curtis, 2014; Buck et al., 2018). An inherent "disgust response" to fecal matter can serve to reduce contact with parasite infectious stages such as helminth eggs or larvae if their direct detection through odor or other means is difficult (Curtis et al., 2011; Sharp et al., 2015). A heterogenous "landscape of disgust" may thus drive spatial use decisions by potential hosts in response to proxies signaling parasite presence (Weinstein et al., 2018a; Kavaliers et al., 2019). That being said, direct chemical detection of parasites by potential hosts occurs. For instance, larval amphibians respond to dead trematode (parasitic fluke) infectious stages in the water through spatial avoidance (Koprivnikar and Penalva, 2015). This research supports the view that parasite risk can be perceived *via* parasite-derived chemical cues given the absence of other possible signals (e.g., vibrations).

Notably, animals under attack by parasites can emit chemical cues that transmit information about this danger to other individuals. Just as the breach of plant cell membranes can trigger the release of specific chemicals (Fürstenberg-Hägg et al., 2013), similar chemical releases can occur when animal tissues are damaged. The release of "alarm cues" as a result of predator-induced physical damage has been well-studied for certain prey, especially in aquatic habitats, and these alarm cues are readily perceived by conspecifics (see review by Ferrari et al., 2010). Some parasites cause damage to external host tissues that may generate chemical cues, as reported for parasites penetrating fish epidermis containing specialized alarm or club cells (Chivers et al., 2007). Conspecifics react to such chemicals—juvenile trout exhibited distinct behaviors in response to tank water from other trout exposed to a skin-penetrating trematode (Poulin et al., 1999). Beyond perceiving chemical cues associated with attack of a conspecific by parasites attempting to establish, it is also possible to detect individuals with already-established infections through chemosensory means. This has been observed with various animals, including amphibians, crustaceans, and rodents (e.g., Kiesecker et al., 1999; Behringer et al., 2006; Kavaliers et al., 2020).

Pre-contact, potential hosts can likely detect the presence of parasites in other ways. For instance, larval amphibians avoid areas with live infectious stages of trematode parasites, possibly detecting water vibrations generated by these free-swimming parasites in addition to potential chemical cues (Rohr et al., 2009). There are also reports of terrestrial animals reacting to parasites in the absence of contact where the sensory mechanisms involved are unknown. As an example, fruit flies (*Drosophila*) respond to the mere presence of parasitic mites separated by a fine mesh screen, but it is not clear whether these enemies are detected through chemical or visual means (Horn and Luong, 2018).

Physical contact with parasites during the pre-consumption phase, or in the initial stages of consumption, is thought to be perceived by potential animal hosts through various tactile means (see review by Kupfer and Fessler, 2018). Contact with ectoparasites, such as ticks and mites, quickly results in behavioral responses in a variety of animal species, from insects to mammals (see reviews by Zhukovskaya et al., 2013; Hart and Hart, 2018). Such immediate behavioral responses also occur in response to penetrating endoparasites. For example, larval amphibians respond to initial contact with free-swimming trematode infectious stages by engaging in characteristic defensive behaviors (e.g., Koprivnikar et al., 2006).

So, how do plants and animals compare in their perception of risk cues associated with their respective herbivore and parasite natural enemies? Chemical cues clearly play a large role for plants pre- and post-contact, and compound-specific attributes may allow plants to perceive specific risk associated with particular herbivores (Agrawal, 2005; Duran-Flores and Heil, 2016). Importantly, herbivores often exhibit species-specific feeding preferences (Bernays and Graham, 1988), and can be grouped into different feeding guilds (e.g., chewers vs. piercers). Plants respond differently to herbivores from different feeding guilds (Ali and Agrawal, 2012), and stronger responses to oviposition by specialist vs. generalist herbivores have been reported (Pashalidou et al., 2013). HIPVs released by plants under attack seemingly convey specific information as to the nature of the threat (Kant et al., 2009; Ameye et al., 2018). Other plants "eavesdropping" on these chemicals may therefore fine-tune their induced response(s) (Kant et al., 2009), and specific HIPVs may selectively attract appropriate enemies of the attacking herbivore (De Moraes et al., 1998).

Like plants and herbivory risk, various animals perceive parasite threat through chemical cues (Behringer et al., 2018; Kavaliers et al., 2020), although most studies to date have focused on particular host taxa, especially nematodes, honeybees, amphibians, and rodents (Sarabian et al., 2018). There is also evidence that animals, like plants, may be able to gauge more subtle aspects of risk through chemosensory means, such as parasite transmission potential and host compatibility (Sharp et al., 2015). For instance, uninfected guppies avoided both chemical and visual cues associated with conspecifics harboring an ectoparasite only in the later stages of infection when direct transmission was most likely (Stephenson et al., 2018).

In studying risk-associated chemical cues, it is also important to consider receptors to highlight the molecular and physiological pathways that are engaged in eliciting responses, and to further elucidate which taxa have evolved specific capability of perceiving enemy risk through chemosensory means. For plants, a receptor has been identified for only one HIPV (ethylene), and finding others represents a key area of further study (Ameye et al., 2018; Karban, 2020). Similarly, the olfactory structures involved with animal detection of parasite-associated chemical cues have been identified only in a few species, primarily rodents and fish (e.g., Wisenden, 2014; Boillat et al., 2015).

Although plants and animals are unlikely to share actual compounds and receptors involved in perceiving enemy risk, broad comparisons of these two victims are still possible to consider the ecological and evolutionary context for features of reliable enemy cues. These comparisons suggest a number of interesting questions, some of which we propose below:

1. For plant-herbivore and animal host-parasite systems, are cues from the enemy or those from attacked conspecifics more readily perceived and/or influential in eliciting responses?
2. Spatiotemporal aspects also are important to consider. Are chemical cues more reliable if they persist in space and time, or are they more effective in conveying immediate risk if they degrade relatively quickly, as seen with certain predation-related cues (e.g., Ferrari et al., 2007)?
3. Other key factors involve the nature of information conveyed by chemical signals of enemy risk. Do cues generated by plant or animal conspecifics experiencing attack or consumption tend to be general indicators of physical damage, or are they specific to the natural enemy?
4. Do victim responses to herbivore or parasite risk generally require a minimum threshold concentration to be exceeded, as with predator cues (Harvell, 1990)? In addition, is this risk perceived in a concentration-dependent manner? This is seen for induced resistance by plants in response to direct damage by herbivores (e.g., Underwood, 2000).
5. The potential audience for enemy risk-associated chemicals (generated by the enemy itself, or victims experiencing attack/consumption) is also a consideration. Are these cues primarily perceived by members of the same species, or by others as well, be they potential victims or natural enemies? Cues with a narrow audience should have more limited scope for NCEs or TMIEs.
6. Are there temporal changes in cue generation or reception? For instance, receivers could become acclimated to parasite or herbivore risk-associated cues rather than remain in a prolonged state of heightened response, or become conditioned to respond faster—both possibilities have been reported for predation-associated cues (e.g., Vilhunen, 2006; Imre et al., 2016).
7. Natural enemies like parasites should benefit from being as cryptic as possible before attacking (Poulin, 2007). Is there selective pressure for any detectable chemical cues to change, and thereby complicate perception by potential victims, as seen for coevolutionary dynamics between social parasites and their victims (e.g., Brandt et al., 2005)?

Enemy Risk-Induced Trait Responses as Defensive Responses

Although resistance against natural enemies can involve constitutive and/or induced traits, only the latter are of relevance for causing NCEs, i.e., there are specific fitness costs associated with expressing phenotypic plasticity as a response to changed risk (Peacor et al., 2020). Induced trait changes encompass a broad suite of strategies used by potential victims to reduce enemy encounter, and/or the likelihood of a successful attack resulting in consumption. While the latter represent adaptive responses that may have associated costs, there is presumably a net benefit; however, maladaptive responses are also possible if the overall costs outweigh the benefits (e.g., Sih et al., 2010; Orrock et al., 2015). For instance, predator-induced increases in prey stress-associated hormones could be beneficial in some ways as a pre-emptive defense (e.g., readiness to fight or flee; Sapolsky et al., 2000), but not in others, such as chronic hormone elevations that take a physiological toll and reduce reproduction (e.g., Sheriff et al., 2009).

Here, we follow the standardized framework and terminology suggested by Peacor et al. (2020) for induced trait responses of prey to predation risk (RITRs), but include herbivores and parasites within this context in terms of potential effects on their victims without or prior to consumption. Critically, enemy RITRs are not interchangeable with NCEs. Rather, RITRs brought about by victim perception of increased natural enemy threat can result in NCEs if these reduce victim abundance or fitness through direct means (e.g., energy reallocation), or affect victim interactions with others in ways that reduce fitness indirectly (Peacor et al., 2020). In other words, all NCEs require costly RITRs, but not all RITRs lead to NCEs if these costs do not demonstrably reduce victim fitness or abundance. Additionally, victim trait alterations could affect the fitness and abundance of a third species, with possible cascading effects involving yet other species; these latter two consequences represent TMIEs (Peacor et al., 2020). To compare the potential for herbivores and parasites to cause NCEs for their plant and animal victims, respectively, it is thus necessary to first consider the scope and nature of RITRs.

We consider research on animal hosts first to highlight a major contrast with plant responses; namely, studies of induced responses to a perceived threat of parasitism have heavily focused on behavioral resistance. These defensive behaviors include those that reduce parasite encounter, or remove parasites before they can establish and incur costs (see reviews by Hart, 1990; Hart and Hart, 2018). Spatial avoidance behavior of hosts to parasite infectious stages, or to proxies such as feces, is well-documented.

For instance, grazing mammals forego foraging near patches containing fecal matter (see reviews by Hutchings et al., 2000; Coulson et al., 2018), especially if the fecal material is relatively "fresh" (e.g., Hutchings et al., 1998). Such avoidance behavior can be well-matched to the species' risk of infection by the parasites in question (Sharp et al., 2015; Weinstein et al., 2018b). There are many excellent examples of behavioral avoidance of parasites by animals (see reviews by Hart, 1990; Behringer et al., 2018); we have limited our discussion of this trait expression here given that adult plants obviously have little capacity for movement in order to avoid contact with herbivores.

Post-contact behavioral resistance by animals is also common, especially for those under attack by ectoparasites (Hart and Hart, 2018). Grooming to remove these parasites before they can establish a prolonged consumptive relationship is a particularly important defensive behavior. Self-grooming is seen in many potential hosts, from insects to mammals and birds (Mooring et al., 2004; Zhukovskaya et al., 2013; Bush and Clayton, 2018), and allo-grooming by members of the same species is also seen in various taxa (e.g., Akinyi et al., 2013). Also, interspecific mutualisms can be critical for ectoparasite removal. For instance, cleaner fish and shrimp are highly effective at removing a variety of ectoparasites from their clients (reviewed by Vaughan et al., 2017), with clients spending a considerable amount of time engaged in cleaning activities if heavily infected (e.g., Grutter, 2001). Here, the behavior of the host is critical in moving to, or remaining at, a cleaning station. Conversely, plants recruit their mutualists (enemies of herbivores) through chemical cues, and not by moving to them (see below).

Compared to parasite risk-induced behavioral changes, there are fewer studies of morphological or physiological trait alterations in animal hosts. As a result, relatively less is known about potential changes in such traits, but recent investigations are broadening our knowledge. For instance, the simple presence of parasites in the vicinity has been reported to alter metabolic activity in potential hosts. *Drosophila* exposed to parasite mites separated by a screen (i.e., no contact possible) increased their metabolic rate (Luong et al., 2017), and this was also seen in fish exposed to motile trematode infectious stages (Nadler et al., 2021). Conversely, larval amphibians with non-contact exposure to trematodes did not exhibit changes in hormones associated with a stress response (Marino et al., 2014).

In contrast to animals and parasite risk, it is not surprising that studies of RITRs in plants primarily involve morphology or physiology given the limited scope of plants for behavioral resistance in response to increased herbivory risk. Of these, changes in the levels of endogenous chemicals representing plant secondary metabolites (PSMs) are well-known (Chen, 2008; Carmona et al., 2011). These metabolites encompass a wide array of compounds, but many (e.g., alkaloids and terpenes) defend against herbivory *via* deterrent or toxic effects (reviews by Bennett and Wallsgrove, 1994; Fürstenberg-Hägg et al., 2013; Erb and Kliebenstein, 2020). In response to cues signaling early or imminent attack by herbivores (see preceding subsection), specific physiological pathways are rapidly activated in plants. The phytohormones jasmonic acid and salicylic acid play key roles, along with the VOC ethylene, triggering the transcription of various defense-related genes (detailed reviews by Howe and Jander, 2008; Fürstenberg-Hägg et al., 2013; Kant et al., 2015).

Beyond induced changes in chemicals such as secondary metabolites, plants that perceive an elevated risk of herbivory can initiate morphological trait alterations that increase their resistance. These include increasing leaf toughness and producing more trichomes to limit herbivore contact through mechanical interference (Fürstenberg-Hägg et al., 2013; Kant et al., 2015). However, it is important to note that cues signaling a threat of herbivory may not result in immediate trait changes, but rather, cause "priming" that allows for a rapid response if the threat continues or an actual attack begins (see Frost et al., 2008; Karban, 2011). This is similar to the "immune-priming" shown by insects if they are exposed to, but not successfully infected by, parasites (reviewed by Sheehan et al., 2020), or in response to cues signaling heightened infection risk, such as crowded conditions, a.k.a. density-dependent prophylaxis (see Wilson and Cotter, 2009).

While plants cannot engage in behavioral resistance to avoid herbivore encounter (but see Dicke, 2009), they still have effective ways to remove these before significant damage occurs. Just like certain animals trying to rid themselves of ectoparasites by soliciting assistance, plants can do so by involving another species. Notably, HIPVs released in response to herbivore attack are not only perceived by other plants, but are known to serve as signals to enemies of those herbivores, including parasitoids and predators (see reviews by Aljbory and Chen, 2018; Pearse et al., 2020). Such interactions among plants, herbivores, and enemies of herbivores represent well-established examples of enemy-related trophic cascades (see Turlings and Erb, 2018). Removal of herbivores by soliciting another species could thus be considered as a type of induced behavioral response by plants.

In highlighting key findings related to enemy RITRs for plant-herbivore and animal host-parasite systems, it is obvious that the traits primarily considered to date differ considerably, i.e., principally behaviors for animals, and morphology or physiology for plants. However, broad patterns can be informative when considering the potential for RITRs to cause NCEs; here we highlight select questions regarding relationships among defense components:

1. Do potential hosts that invest relatively heavily in constitutive resistance (e.g., high innate immunity) exhibit reduced capacity in terms of parasite RITRs? Some studies have reported trade-offs in plants with respect to their investment in constitutive vs. induced resistance (see Kempel et al., 2011). The extent to which this occurs for animals is not clear (e.g., Klemme et al., 2020; Schreier and Grindstaff, 2020), but could have significance for NCEs. Host life history and parasite characteristics (e.g., virulence) are also expected to affect the relative cost(s) of investment in constitutive versus induced defense (Boots and Best, 2018) – these additional factors may have implications for expression of RITRs. Intra- and interspecific variation in resistance investment could thus influence the potential for parasites to cause NCEs

depending on whether there is limited or expanded scope for parasite RITRs.

2. Are there relationships among enemy RITRs for both victim systems? Plants have been described as "jacks of all trades, and masters of all trades" when it comes to trade-offs among induced traits (Koricheva et al., 2004), i.e., they apparently exhibit few obvious limitations in this context (Karban, 2011). In fact, plants often exhibit "defense syndromes" that consist of suites of positively-correlated response traits (Agrawal and Fishbein, 2006). Whether there are associations among parasite RITRs in animals remains largely unexplored.
3. If suites of RITRs are observed, are NCEs more detectable? Notably, **allocation, opportunity, or ecological costs** (see **Glossary**) might be higher if RITRs are positively correlated. Parasite RITRs could have negative or positive underlying relationships among themselves, thereby affecting the potential for NCEs and TMIEs to result. Such underlying trait covariation has been identified as an important means by which parasites can alter the behaviors of infected hosts in complex ways (Poulin, 2013).

Non-consumptive Effects of Natural Enemies

As detailed above, enemy RITRs must cause reductions in victim fitness or abundance to be considered as NCEs (Peacor et al., 2020). Based on these criteria, surprisingly few studies have actually demonstrated NCEs related to natural enemy presence, even for predator-prey interactions (Sheriff et al., 2020b). Various logistical hurdles make it difficult to directly assess how natural enemy presence affects victim fitness (reproduction, recruitment, and mortality), and especially abundance, for most systems (Sheriff et al., 2020b). However, inconsistency in terminology also poses problems because "non-consumptive effect" has been widely used to describe a trait change in a potential victim that occurs prior to actual meaningful consumption by a natural enemy (Peacor et al., 2020). In the absence of direct measures of victim fitness or abundance when evaluating the costs(s) of enemy RITRs, the strongest case for potential NCEs can thus be made when evaluating traits that serve as good proxies for fitness (e.g., condition or growth rate—Sheriff et al., 2020b).

Many studies have examined whether plants experience fitness reductions as a result of induced defensive responses to actual herbivory; however, those studies often consider costs by measuring traits correlated with fitness, especially growth or development (e.g., plant size and seed germination), or physiological aspects such as photosynthesis (see Cipollini et al., 2003; Züst and Agrawal, 2017). When evaluating herbivore NCEs on plants in response to heightened risk alone (i.e., no consumption), these same correlative traits should be relevant for inferring potential fitness costs. For instance, the growth of maize seedlings was significantly reduced if these were exposed to GLVs emitted by neighbors (Engelberth and Engelberth, 2019). In contrast, wild tobacco plants exposed to HIPVs emanating from damaged sagebrush actually produced more seeds relative to controls (Karban and Maron, 2002). This latter response might have been a form of terminal investment preceding herbivory.

Costs may occur through direct means, such as energy reallocation to herbivore resistance, or manifest as ecological or opportunity costs by altering inter- or intraspecific interactions (Cipollini et al., 2003). Notably, plants eavesdropping on HIPVs may undergo trait changes that make them inferior or superior competitors against the same or different species (reviewed by Dicke and Baldwin, 2010). Herbivore RITRs, such as increased lignin content after plant exposure to GLVs, can also affect plant susceptibility to pathogens and parasites (e.g., Kishimoto et al., 2006), thereby influencing plant fitness.

In terms of parasite RITRs in animals, very few studies have reported direct reductions in fitness or abundance. As previously mentioned, *Drosophila* show increased metabolic rates in the presence of physically-separated parasitic mites, and this caused reductions in both fly fecundity and longevity (Horn and Luong, 2018). Overall, it is likely that fitness costs (i.e., NCEs) are associated with changes in victim traits if the latter involve substantial energy use that cannot be compensated for, such as through increased feeding. With this in mind, it is probable that behavioral RITRs to parasite threat are associated with fitness costs. As individuals of many species forego foraging opportunities in order to reduce their chances of parasite encounter (e.g., Fritzsche and Allan, 2012; Koprivnikar and Penalva, 2015; Weinstein et al., 2018b), this avoidance may reduce energy intake—similar to predator effects (Peacor et al., 2020). More work is needed to determine whether such avoidance behaviors often have negative effects on direct fitness measures or reasonable proxies.

The fitness costs of enemy RITRs have been more straightforward to evaluate from plants than from animals. Direct negative effects on plant reproduction or population abundance as a result of herbivore RITRs have been reported (e.g., Yip et al., 2019), but net benefits have also been seen, such as for neighboring plants exposed to HIPVs (Karban et al., 2012). Fitness consequences in plants are easier to infer because good proxies are available (Cipollini et al., 2003). In contrast, relatively few studies have demonstrated parasite RITRs translating into NCEs for animals. Future investigations should specifically consider reasonable fitness correlates of RITRs, similar to plant-herbivore studies.

Trait-Mediated Indirect Effects

Our focus thus far has been on enemy RITRs in plant-herbivore and animal parasite-host systems in terms of their potential to cause NCEs, but we briefly note ways in which trait changes could have community-level consequences by affecting victim interactions with other species, i.e., trait-mediated indirect effects (TMIEs). Potential TMIEs related to parasite risk have been reviewed (Buck and Ripple, 2017), thus we limit our discussion here to noting the general pathways involved, and how these compare for plants and herbivores. One way in which parasite risk affects a third species is when altered foraging behavior by potential victims increases food resources for other consumers, especially competing species (Buck et al., 2018). For example, invasive ant species are often successful by dominating food resources through aggression; however, the presence of parasitoids that use the invasive ants as hosts alters the invaders' foraging behaviors such that competing endemic ant species are no longer at a disadvantage (Feener, 2000).

When it comes to TMIEs broadly involving plant-herbivore interactions, most work has considered reductions in plant damage owing to altered herbivore behaviors when the natural enemies of these herbivores (predators and parasitoids) are recruited (e.g., Culshaw-Maurer et al., 2020). But enemy RITRs in plants themselves could also result in TMIEs. For instance, the negative effects of stinkbug predators on hornworm caterpillars were reduced if the latter consumed tomato plants that were induced to express high levels of jasmonate (Kaplan and Thaler, 2010). HIPVs released in response to herbivore attack are not only subject to eavesdropping by other plants, or useful in recruiting herbivore enemies—these cues can attract other herbivore species to the area, with implications for neighboring plants and herbivore competitive interactions (reviewed by Dicke and van Loon, 2000). Remarkably, plant HIPVs can even affect the reproduction of nearby herbivores by suppressing pheromone detection and mate location (Hatano et al., 2015). Lastly, different plant species can exhibit divergent trait changes when exposed to the same HIPVs, with either increased or decreased fitness, thereby influencing plant community composition (Freundlich and Frost, 2019).

CROSS-SYSTEM COMPARISONS AND FUTURE DIRECTIONS

We have compared plant-herbivore and animal host-parasite systems in terms of victim-enemy interactions toward identifying broad attributes relevant for documenting NCEs—similar to efforts to draw parallels between host-parasite and prey-predator systems (e.g., Raffel et al., 2008; Buck et al., 2018; Daversa et al., 2021), and plant-herbivore and prey-predator systems (Sheriff et al., 2020a). Specifically, we considered plant-herbivore and animal-parasite systems with respect to the victim's ability to perceive enemy risk, the range and magnitude of possible RITRs shown by victims, and whether these responses affect victim fitness or abundance (NCEs). Below we explicitly highlight areas where plant-herbivore and animal host-parasite work could potentially and particularly inform each another.

Post-contact but Pre-consumption Responses and Costs?

One way in which plant-herbivore and animal host-parasite systems are distinct from prey-predator is that the latter has relatively distinct pre-consumption and consumption phases of the interaction, and limited potential for different avenues of adaptation by the victim (i.e., prey must engage in pre-emptive defenses or risk having a fitness of zero). In contrast, plant and animal victims of herbivores and parasites, respectively, can experience a "gray zone" in terms of a post-contact time period during which NCEs may occur while their enemies try to establish feeding, and have not yet altered host energy balance (Buck and Ripple, 2017). In other words, NCEs could frequently occur post-contact for these two natural enemy systems, whereas this seems unlikely for prey-predator interactions (i.e., consumption begins immediately after contact). Given this possibility for post-contact NCEs, it will be important to standardize whether the definition of NCEs should be restricted to pre-encounter influences by parasites and herbivores. To aid in this, it would be very useful to conduct studies that specifically look for costly trait alterations during this gray zone period, as well as develop some criteria to define a shift from NCEs to CEs (Buck and Ripple, 2017). Such criteria will have to include the extent to which the trait response can be viewed as defensive (i.e., adaptive) rather than simply a side effect of consumption.

Heavy Reliance on Chemical Cues to Perceive Enemy Risk?

When considering how plants and animals may perceive their herbivore and parasite enemies, respectively, it would appear that both victim types commonly rely on chemical cues which either directly emanate from the enemy (kairomones), or are released by conspecifics under attack (alarm cues). Because invertebrate herbivores and parasite infectious stages are likely difficult for their victims to perceive through audiovisual cues, as compared to prey which often can detect predators in this manner (Brown et al., 1999; Wirsing et al., 2021), reliance on chemical signals in both victim systems is not surprising. Notably, many studies of parasite avoidance behavior in animals have been conducted with aquatic species, and chemical cues are particularly well-suited for this type of environment (Behringer et al., 2018). Terrestrial animals also could be adept at detecting infection risk through chemical means, thus the greater number of examples from aquatic systems may reflect relative study effort rather than say anything about a victim's ability based on its habitat. Further work with terrestrial animal host-parasite systems is necessary for a broader comparison to chemical cues used by plants for herbivore detection.

Do All Measurable RITRs Translate Into NCEs?

In terms of enemy RITRs, the focus to date has been on behavioral alterations shown by animals in response to perceived parasite threat. For obvious reasons, studies of trait changes in plants have instead considered various aspects of morphology and physiology. Some of these are directly relevant for evaluating the cost(s) of induced resistance in terms of reduced victim fitness or abundance, with other measures often serving as good proxies (e.g., plant growth or development). It will be essential to obtain similarly relevant measures for animals in order to determine how parasites may exact costs irrespective of actual consumption. This represents a particularly critical and urgent area for future work.

Can Molecular and Synthetic Approaches Be Informative?

Studies of plant-herbivore interactions are increasingly utilizing genetic and molecular approaches to examine how cues signaling enemy risk may translate into trait changes (Johnson, 2011; Züst and Agrawal, 2017). Such tools for animal host-parasite work would be very useful, not only to better understand the mechanistic pathways involved in RITRs, but also potential alterations of many traits at once (e.g., *via* pleiotropic genes). As plants often exhibit "defense syndromes" for resistance-related traits (Agrawal and Fishbein, 2006), such possibilities should be investigated for parasite RITRs in animals. Using "synthetic" means by which to induce herbivore risk-related responses in plants has been an important tool in this area of study (Cipollini et al., 2003; Howe and Jander, 2008)—this approach could prove useful for examining animal responses to parasite risk if the precise cues used to gauge risk can be identified. Animal host-parasite work should further consider variation among natural populations in terms of RITRs, as well as potential differences between domesticated and wild species—both of which have been explored with plants (Cipollini et al., 2003; Züst and Agrawal, 2017).

Importance of Colonial or Group-Living Lifestyles?

For both natural enemy risk perception and RITRs, there may be particular value in comparing plant-herbivore and animal-host parasite systems comprised of colonies, particularly if individuals are kin-related. Various woody and herbaceous flowering plants can form clonal colonies (Fischer and Van Kleunen, 2001). While clonal colonies of animals are less common (e.g., some sponges and corals), social insects have a high degree of relatedness, and engage in a number of cooperative hygienic behaviors to protect the colony against parasites and pathogens (Wilson-Rich et al., 2009). In such cases, it would be valuable for related conspecifics to quickly perceive and respond to chemical cues released by individuals under attack. What might otherwise be considered as "eavesdropping" by other individuals so as to gain a defensive advantage could actually be beneficial (Karban, 2011), making this type of enemy-associated cue particularly important in the context of RITRs and subsequent NCEs. Group-living plants and animals, especially those often surrounded by close kin with similar vulnerability, may thus be particularly sensitive to conspecific-generated cues signaling enemy presence, and also more likely to display RITRs.

Do Sessile Lifestyles Select for Certain Mechanisms and Pathways?

When considering the chain of events leading to NCEs, sessile animals (e.g., bivalves) may particularly share key features with plants in terms of how these two victim types perceive parasitism or herbivory risk, respectively. Such comparisons have proposed for their anti-predator defenses (Sheriff et al., 2020a), and likely apply to other natural enemies. Notably, many sessile animals have limited audiovisual sensory capability; just like plants, this may make them relatively reliant on chemical cues signaling parasite risk, or especially sensitive to even slight contact. For instance, blue mussels quickly retract their filtering siphons and close their shells in the presence of free-swimming trematode infectious stages, but it is not clear to which enemy-related cues they react (Selbach and Mouritsen, 2020).

Throughout, we discussed the likelihood of documenting costly pre-emptive defenses through trait changes in response to natural enemy cues in two victim systems (plant-herbivore and animal-parasite). We showed that enemy cues in those two systems are seemingly detected, and invoke RITRs that can be considered as pre-emptive defense. We currently do not know how frequent or costly these are relative to RITRs of prey, or to consumption-induced trait changes of the same two victim systems under study. Costly pre-emptive defenses by prey, and consumption-based defensive responses of plants and animal hosts to herbivores and parasites, respectively, are well-documented in comparison. Future work should thus consider the interplay between pre-emptive (risk-induced) and consumption-induced trait changes in the two victim systems, particularly as it relates to applying NCE knowledge for natural enemy control (e.g., Culshaw-Maurer et al., 2020), and the population viability of focal organisms in habitats where herbivore or parasite threat may be either diminished or heightened (e.g., Rusch et al., 2013; Gottdenker et al., 2014).

Looking for both parallels and contrasts between different enemy-victim systems is valuable to advance the general field of natural enemy ecology, especially for understanding the importance of NCEs for victim populations and the importance of TMIEs for affecting communities. NCEs may be more common in particular victim-enemy systems based on their shared inherent biological potential for perceiving and responding to altered risk; however, certain ecological or evolutionary factors also could drive NCE occurrence. For instance, co-evolved species could be more likely to exhibit NCEs than novel consumer-victim systems, as seen for predator-prey interactions (Sih et al., 2010). Valuable insights regarding natural enemy ecology, especially the "ecology of fear," have been gained by integrating concepts and knowledge for animal host-parasite and prey-predator interactions (e.g., Raffel et al., 2008; Buck et al., 2018; Weinstein et al., 2018a; Daversa et al., 2021). By broadly synthesizing and contrasting key aspects of plant and animal trait responses to the risks posed by herbivores and parasites, respectively, we hope that similar benefits have been seen in terms of advancing our understanding of NCEs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (Grant Nos. 04622 for JK and 100118 for MF).

ACKNOWLEDGMENTS

Silhouettes of organisms in **Figure 1** were obtained from PhyloPic (http://phylopic.org/) and used under a Creative Commons license. We also thank the reviewers for their constructive comments to improve the original manuscript.

REFERENCES

Adamson, M. L., and Caira, J. N. (1994). Evolutionary factors influencing the nature of parasite specificity. *Parasitology* 109, S85–S95.

Agrawal, A. A. (2005). Future directions in the study of induced plant responses to herbivory. *Entomol. Exp. Appl.* 115, 97–105. doi: 10.1111/j.1570-7458.2005.00294.x

Agrawal, A. A., and Fishbein, M. (2006). Plant defense syndromes. *Ecology* 87, S132–S149. doi: 10.1111/ens.12172

Agrawal, A. A., and Fishbein, M. (2008). Phylogenetic escalation and decline of plant defense strategies. *Proc. Natl. Acad. Sci. USA* 105, 10057–10060. doi: 10.1073/pnas.0802368105

Akinyi, M. Y., Tung, J., Jeneby, M., Patel, N. B., Altmann, J., and Alberts, S. C. (2013). Role of grooming in reducing tick load in wild baboons (*Papio cynocephalus*). *Anim. Behav.* 85, 559–568. doi: 10.1016/j.anbehav.2012.12.012

Ali, J. G., and Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends Plant Sci.* 17, 293–302. doi: 10.1016/j.tplants.2012.02.006

Aljbory, Z., and Chen, M. S. (2018). Indirect plant defense against insect herbivores: a review. *Insect Sci.* 25, 2–23. doi: 10.1111/1744-7917.12436

Ameye, M., Allmann, S., Verwaeren, J., Smagghe, G., Haesaert, G., Schuurink, R. C., et al. (2018). Green leaf volatile production by plants: a meta-analysis. *New Phytol.* 220, 666–683. doi: 10.1111/nph.14671

Baucom, R. S., and de Roode, J. C. (2011). Ecological immunology and tolerance in plants and animals. *Funct. Ecol.* 25, 8–28.

Behringer, D. C., Butler, M. J., and Shields, J. D. (2006). Ecology: avoidance of disease by social lobsters. *Nature* 441:421. doi: 10.1038/441421a

Behringer, D. C., Karvonen, A., and Bojko, J. (2018). Parasite avoidance behaviors in aquatic environments. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170202. doi: 10.1098/rstb.2017.0202

Bennett, R. N., and Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytol.* 127, 617–633. doi: 10.1111/j.1469-8137.1994.tb02968.x

Bernays, E., and Graham, M. (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology* 69, 886–892. doi: 10.2307/1941237

Boillat, M., Challet, L., Rossier, D., Kan, C., Carleton, A., and Rodriguez, I. (2015). The vomeronasal system mediates sick conspecific avoidance. *Curr. Biol.* 25, 251–255. doi: 10.1016/j.cub.2014.11.061

Boots, M., and Best, A. (2018). The evolution of constitutive and induced defences to infectious disease. *Proc. R. Soc. Lond. B Biol. Sci.* 285:20180658. doi: 10.1098/rspb.2018.0658

Bouwmeester, H., Schuurink, R. C., Bleeker, P. M., and Schiestl, F. (2019). The role of volatiles in plant communication. *Plant J.* 100, 892–907. doi: 10.1111/tpj.14496

Brandt, M., Heinze, J., Schmitt, T., and Foitzik, S. (2005). A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. *J. Evol. Biol.* 18, 576–586. doi: 10.1111/j.1420-9101.2004.00867.x

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. doi: 10.2307/1383287

Buck, J. C., and Ripple, W. J. (2017). Infectious agents trigger trophic cascades. *Trends Ecol. Evol.* 32, 681–694. doi: 10.1016/j.tree.2017.06.009

Buck, J. C., Weinstein, S. B., and Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends Ecol. Evol.* 33, 619–632. doi: 10.1016/j.tree.2018.05.001

Bush, S. E., and Clayton, D. H. (2018). Anti-parasite behavior of birds. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170196. doi: 10.1098/rstb.2017.0196

Carmona, D., Lajeunesse, M. J., and Johnson, M. T. (2011). Plant traits that predict resistance to herbivores. *Funct. Ecol.* 25, 358–367. doi: 10.1111/j.1365-2435.2010.01794.x

Chen, M. S. (2008). Inducible direct plant defense against insect herbivores: a review. *Insect Sci.* 15, 101–114. doi: 10.1111/j.1744-7917.2008.00190.x

Chivers, D. P., Wisenden, B. D., Hindman, C. J., Michalak, T. A., Kusch, R. C., Kaminskyj, S. G., et al. (2007). Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: possible defense against pathogens, parasites and UVB radiation. *Proc. R. Soc. Lond. B Biol. Sci.* 274, 2611–2619. doi: 10.1098/rspb.2007.0709

Cipollini, D., Purrington, C. B., and Bergelson, J. (2003). Costs of induced responses in plants. *Basic Appl. Ecol.* 4, 79–89. doi: 10.1078/1439-1791-00134

Combes, C. (2001). *Parasitism: the Ecology and Evolution of Intimate Interactions*. Chicago: University of Chicago Press.

Cortez, M. H., and Duffy, M. A. (2020). Comparing the indirect effects between exploiters in predator-prey and host-pathogen systems. *Am. Nat.* 196, E144–E159.

Coulson, G., Cripps, J. K., Garnick, S., Bristow, V., and Beveridge, I. (2018). Parasite insight: assessing fitness costs, infection risks and foraging benefits relating to gastrointestinal nematodes in wild mammalian herbivores. *Philos. Trans. R. Soc. B Biol. Sci.* 373:0170197.

Culshaw-Maurer, M., Sih, A., and Rosenheim, J. A. (2020). Bugs scaring bugs: enemy-risk effects in biological control systems. *Ecol. Lett.* 23, 1693–1714. doi: 10.1111/ele.13601

Curtis, V. A. (2014). Infection-avoidance behavior in humans and other animals. *Trends Immunol.* 35, 457–464. doi: 10.1016/j.it.2014.08.006

Curtis, V. A., De Barra, M., and Aunger, R. (2011). Disgust as an adaptive system for disease avoidance behavior. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 389–401. doi: 10.1098/rstb.2010.0117

Dahl, E., Orizaola, G., Winberg, S., and Laurila, A. (2012). Geographic variation in corticosterone response to chronic predator stress in tadpoles. *J. Evol. Biol.* 25, 1066–1076. doi: 10.1111/j.1420-9101.2012.02493.x

Daversa, D. R., Hechinger, R. F., Madin, E., Fenton, A., Dell, A. I., Ritchie, E. G., et al. (2021). Broadening the ecology of fear: non-lethal effects arise from diverse responses to predation and parasitism. *Proc. R. Soc. Lond. B Biol. Sci.* 288:20202966. doi: 10.1098/rspb.2020.2966

De Moraes, C. M., Lewis, W. J., Pare, P. W., Alborn, H. T., and Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. *Nature* 393, 570–573. doi: 10.1038/31219

Dicke, M. (2009). Behavioral and community ecology of plants that cry for help. *Plant Cell Environ.* 32, 654–665. doi: 10.1111/j.1365-3040.2008.01913.x

Dicke, M., and Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends Plant Sci.* 15, 167–175. doi: 10.1016/j.tplants.2009.12.002

Dicke, M., and van Loon, J. J. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.* 97, 237–249. doi: 10.1046/j.1570-7458.2000.00736.x

Duran-Flores, D., and Heil, M. (2016). Sources of specificity in plant damaged-self recognition. *Curr. Opin. Plant Biol.* 32, 77–87. doi: 10.1016/j.pbi.2016.06.019

Engelberth, J., and Engelberth, M. (2019). The costs of green leaf volatile-induced defense priming: temporal diversity in growth responses to mechanical wounding and insect herbivory. *Plants* 8:e23.

Erb, M., and Kliebenstein, D. J. (2020). Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. *Plant Physiol.* 184, 39–52. doi: 10.1104/pp.20.00433

Falk, K. L., Kästner, J., Bodenhausen, N., Schramm, K., Paetz, C., Vassão, D. G., et al. (2014). The role of glucosinolates and the jasmonic acid pathway in resistance of *Arabidopsis thaliana* against molluscan herbivores. *Mol. Ecol.* 23, 1188–1203. doi: 10.1111/mec.12610

Feener, D. H. (2000). Is the assembly of ant communities mediated by parasitoids? *Oikos* 90, 79–88. doi: 10.1034/j.1600-0706.2000.900108.x

Ferrari, M. C., Messier, F., and Chivers, D. P. (2007). Degradation of chemical alarm cues under natural conditions: risk assessment by larval woodfrogs. *Chemoecology* 17, 263–266. doi: 10.1007/s00049-007-0381-0

Ferrari, M. C., Wisenden, B. D., and Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Can. J. Zool.* 88, 698–724. doi: 10.1139/z10-029

Fischer, M., and Van Kleunen, M. (2001). On the evolution of clonal plant life histories. *Evol. Ecol.* 15, 565–582. doi: 10.1023/a:1016013721469

Freundlich, G. E., and Frost, C. J. (2019). Variable costs and benefits of eavesdropping a green leaf volatile on two plant species in a common garden. *bioRxiv [preprint]*. Available Online at: https://doi.org/10.1101/370692.

Fritzsche, A., and Allan, B. F. (2012). The ecology of fear: host foraging behavior varies with the spatio-temporal abundance of a dominant ectoparasite. *EcoHealth* 9, 70–74. doi: 10.1007/s10393-012-0744-z

Frost, C. J., Mescher, M. C., Carlson, J. E., and De Moraes, C. M. (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiol.* 146, 818–824. doi: 10.1104/pp.107.113027

Fürstenberg-Hägg, J., Zagrobelny, M., and Bak, S. (2013). Plant defense against insect herbivores. *Int. J. Mol. Sci.* 14, 10242–10297. doi: 10.3390/ijms140510242

Gottdenker, N. L., Streicker, D. G., Faust, C. L., and Carroll, C. R. (2014). Anthropogenic land use change and infectious diseases: a review of the evidence. *EcoHealth* 11, 619–632. doi: 10.1007/s10393-014-0941-z

Grutter, A. S. (2001). Parasite infection rather than tactile stimulation is the proximate cause of cleaning behavior in reef fish. *Proc. R. Soc. Lond. B Biol. Sci.* 268, 1361–1365. doi: 10.1098/rspb.2001.1658

Hart, B. L. (1990). Behavioral adaptations to pathogens and parasites: five strategies. *Neurosci. Biobehav. Rev.* 14, 273–294. doi: 10.1016/s0149-7634(05)80038-7

Hart, B. L. (2011). Behavioral defenses in animals against pathogens and parasites: parallels with the pillars of medicine in humans. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 3406–3417. doi: 10.1098/rstb.2011.0092

Hart, B. L., and Hart, L. A. (2018). How mammals stay healthy in nature: the evolution of behaviors to avoid parasites and pathogens. *Philos. Trans. R. Soc. B Biol. Sci.* 373: 20170205. doi: 10.1098/rstb.2017.0205

Harvell, C. D. (1990). The ecology and evolution of inducible defenses. *Q. Rev. Biol.* 65, 323–340. doi: 10.2307/j.ctv1ddd1cn.23

Hatano, E., Saveer, A. M., Borrero-Echeverry, F., Strauch, M., Zakir, A., Bengtsson, M., et al. (2015). A herbivore-induced plant volatile interferes with host plant and mate location in moths through suppression of olfactory signalling pathways. *BMC Biol.* 13:75.

Hilker, M., and Meiners, T. (2006). Early herbivore alert: insect eggs induce plant defense. *J. Chem. Ecol.* 32, 1379–1397. doi: 10.1007/s10886-006-9057-4

Horn, C. J., and Luong, L. T. (2018). Proximity to parasites reduces host fitness independent of infection in a Drosophila–Macrocheles system. *Parasitology* 145, 1564–1569. doi: 10.1017/s0031182018000379

Howe, G. A., and Jander, G. (2008). Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* 59, 41–66. doi: 10.1146/annurev.arplant.59.032607.092825

Hutchings, M. R., Kyriazakis, I., Anderson, D. H., Gordon, I. J., and Coop, R. L. (1998). Behavioral strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. *Anim. Sci.* 67, 97–106. doi: 10.1017/s1357729800009838

Hutchings, M. R., Kyriazakis, I., Papachristou, T. G., Gordon, I. J., and Jackson, F. (2000). The herbivores' dilemma: trade-offs between nutrition and parasitism in foraging decisions. *Oecologia* 124, 242–251. doi: 10.1007/s004420000367

Imre, I., Di Rocco, R. T., Brown, G. E., and Johnson, N. S. (2016). Habituation of adult sea lamprey repeatedly exposed to damage-released alarm and predator cues. *Environ. Biol. Fishes* 99, 613–620. doi: 10.1007/s10641-016-0503-z

Johnson, M. T. (2011). Evolutionary ecology of plant defenses against herbivores. *Funct. Ecol.* 25, 305–311. doi: 10.1111/j.1365-2435.2011.01838.x

Kant, M. R., Bleeker, P. M., Van Wijk, M., Schuurink, R. C., and Haring, M. A. (2009). Plant volatiles in defense. *Adv. Bot. Res.* 51, 613–666.

Kant, M. R., Jonckheere, W., Knegt, B., Lemos, F., Liu, J., Schimmel, B. C. J., et al. (2015). Mechanisms and ecological consequences of plant defense induction and suppression in herbivore communities. *Ann. Bot.* 115, 1015–1051. doi: 10.1093/aob/mcv054

Kaplan, I., and Thaler, J. S. (2010). Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos* 119, 1105–1113. doi: 10.1111/j.1600-0706.2009.18311.x

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* 25, 339–347. doi: 10.1111/j.1365-2435.2010.01789.x

Karban, R. (2020). The ecology and evolution of induced responses to herbivory and how plants perceive risk. *Ecol. Entomol.* 45, 1–9. doi: 10.1111/een.12771

Karban, R., and Maron, J. (2002). The fitness consequences of interspecific eavesdropping between plants. *Ecology* 83, 1209–1213. doi: 10.1890/0012-9658(2002)083[1209:tfcoie]2.0.co;2

Karban, R., Ishizaki, S., and Shiojiri, K. (2012). Long-term demographic consequences of eavesdropping for sagebrush. *J. Ecol.* 100, 932–938. doi: 10.1111/j.1365-2745.2012.01974.x

Karban, R., Orrock, J. L., Preisser, E. L., and Sih, A. (2016). A comparison of plants and animals in their responses to risk of consumption. *Curr. Opin. Plant Biol.* 32, 1–8. doi: 10.1016/j.pbi.2016.05.002

Karban, R., Yang, L. H., and Edwards, K. F. (2014). Volatile communication between plants that affects herbivory: a meta-analysis. *Ecol. Lett.* 17, 44–52. doi: 10.1111/ele.12205

Kavaliers, M., Ossenkopp, K. P., and Choleris, E. (2019). Social neuroscience of disgust. *Genes Brain Behav.* 18:e12508. doi: 10.1111/gbb.12508

Kavaliers, M., Ossenkopp, K. P., and Choleris, E. (2020). Pathogens, odors, and disgust in rodents. *Neurosci. Biobehav. Rev.* 119, 281–293. doi: 10.1016/j.neubiorev.2020.09.037

Kempel, A., Schädler, M., Chrobock, T., Fischer, M., and van Kleunen, M. (2011). Tradeoffs associated with constitutive and induced plant resistance against herbivory. *Proc. Natl Acad. Sci. USA* 108, 5685–5689. doi: 10.1073/pnas.1016508108

Kiesecker, J. M., Skelly, D. K., Beard, K. H., and Preisser, E. (1999). Behavioral reduction of infection risk. *Proc. Natl Acad. Sci. USA* 96, 9165–9168. doi: 10.1073/pnas.96.16.9165

Kishimoto, K., Matsui, K., Ozawa, R., and Takabayashi, J. (2006). Components of C6-aldehyde-induced resistance in Arabidopsis thaliana against a necrotrophic fungal pathogen. *Botrytis cinerea*. *Plant Sci.* 170, 715–723. doi: 10.1016/j.plantsci.2005.11.002

Klemme, I., Hyvärinen, P., and Karvonen, A. (2020). Negative associations between parasite avoidance, resistance and tolerance predict host health in salmonid fish populations. *Proc. R. Soc. Lond. B Biol. Sci.* 287:20200388. doi: 10.1098/rspb.2020.0388

Kollasch, A. M., Abdul-Kafi, A. R., Body, M. J., Pinto, C. F., Appel, H. M., and Cocroft, R. B. (2020). Leaf vibrations produced by chewing provide a consistent acoustic target for plant recognition of herbivores. *Oecologia* 194, 1–13. doi: 10.1007/s00442-020-04672-2

Koprivnikar, J., and Penalva, L. (2015). Lesser of two evils? Foraging choices in response to threats of predation and parasitism. *PLoS One* 10:e0116569. doi: 10.1371/journal.pone.0116569

Koprivnikar, J., Forbes, M. R., and Baker, R. L. (2006). On the efficacy of anti-parasite behavior: a case study of tadpole susceptibility to cercariae of Echinostoma trivolvis. *Can. J. Zool.* 84, 1623–1629. doi: 10.1139/z06-158

Koricheva, J., Nykänen, H., and Gianoli, E. (2004). Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? *Am. Nat.* 163, E64–E75.

Kost, C., and Heil, M. (2006). Herbivore-induced plant volatiles induce an indirect defense in neighbouring plants. *J. Ecol.* 94, 619–628. doi: 10.1111/j.1365-2745.2006.01120.x

Kupfer, T. R., and Fessler, D. M. (2018). Ectoparasite defense in humans: relationships to pathogen avoidance and clinical implications. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170207. doi: 10.1098/rstb.2017.0207

Lafferty, K. D., and Kuris, A. M. (2002). Trophic strategies, animal diversity and body size. *Trends Ecol. Evol.* 17, 507–513. doi: 10.1016/s0169-5347(02)02615-0

Leung, T. L. F. (2014). Fish as parasites: an insight into evolutionary convergence in adaptations for parasitism. *J. Zool.* 294, 1–12. doi: 10.1111/jzo.12148

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. doi: 10.1139/z90-092

Luong, L. T., Horn, C. J., and Brophy, T. (2017). Mitey costly: energetic costs of parasite avoidance and infection. *Physiol. Biochem. Zool.* 90, 471–477. doi: 10.1086/691704

Magalhães, D. M., Da Silva, I. T. F. A., Borges, M., Laumann, R. A., and Blassioli-Moraes, M. C. (2019). Anthonomus grandis aggregation pheromone induces cotton indirect defense and attracts the parasitic wasp *Bracon vulgaris*. *J. Exp. Bot.* 70, 1891–1901. doi: 10.1093/jxb/erz040

Marino, J. A., Holland, M. P., and Middlemis Maher, J. (2014). Predators and trematode parasites jointly affect larval anuran functional traits and corticosterone levels. *Oikos* 123, 451–460. doi: 10.1111/j.1600-0706.2013.00896.x

Mooring, M. S., Blumstein, D. T., and Stoner, C. J. (2004). The evolution of parasite-defense grooming in ungulates. *Biol. J. Linn. Soc. Lond.* 81, 17–37. doi: 10.1111/j.1095-8312.2004.00273.x

Nadler, L. E., Bengston, E., Eliason, E. J., Hassibi, C., Helland-Riise, S. H., Johansen, I. B., et al. (2021). A brain-infecting parasite impacts host metabolism both during exposure and after infection is established. *Funct. Ecol.* 35, 105–116. doi: 10.1111/1365-2435.13695

Orrock, J. L., Sih, A., Ferrari, M. C., Karban, R., Preisser, E. L., Sheriff, M. J., et al. (2015). Error management in plant allocation to herbivore defense. *Trends Ecol. Evol.* 30, 441–445. doi: 10.1016/j.tree.2015.06.005

Pashalidou, F. G., Lucas-Barbosa, D., van Loon, J. J., Dicke, M., and Fatouros, N. E. (2013). Phenotypic plasticity of plant response to herbivore eggs: effects on resistance to caterpillars and plant development. *Ecology* 94, 702–713. doi: 10.1890/12-1561.1

Peacor, S. D., Barton, B. T., Kimbro, D. L., Sih, A., and Sheriff, M. J. (2020). A framework and standardized terminology to facilitate the study of predation-risk effects. *Ecology* 10:e03152.

Peacor, S. D., Peckarsky, B. L., Trussell, G. C., and Vonesh, J. R. (2013). Costs of predator-induced phenotypic plasticity: a graphical model for predicting the contribution of nonconsumptive and consumptive effects of predators on prey. *Oecologia* 171, 1–10. doi: 10.1007/s00442-012-2394-9

Pearse, I. S., LoPresti, E., Schaeffer, R. N., Wetzel, W. C., Mooney, K. A., Ali, J. G., et al. (2020). Generalising indirect defense and resistance of plants. *Ecol. Lett.* 23, 1137–1152. doi: 10.1111/ele.13512

Peiffer, M., Tooker, J. F., Luthe, D. S., and Felton, G. W. (2009). Plants on early alert: glandular trichomes as sensors for insect herbivores. *New Phytol.* 184, 644–656. doi: 10.1111/j.1469-8137.2009.03002.x

Poulin, R. (2007). *Evolutionary Ecology of Parasites*, 2nd Edn. Oxford: Princeton University Press.

Poulin, R. (2013). Parasite manipulation of host personality and behavioral syndromes. *J. Exp. Biol.* 216, 18–26. doi: 10.1242/jeb.073353

Poulin, R., Marcogliese, D. J., and McLaughlin, J. D. (1999). Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *J. Fish Biol.* 55, 47–53. doi: 10.1111/j.1095-8649.1999.tb00655.x

Råberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 37–49. doi: 10.1098/rstb.2008.0184

Raffel, T. R., Martin, L. B., and Rohr, J. R. (2008). Parasites as predators: unifying natural enemy ecology. *Trends Ecol. Evol.* 23, 610–618. doi: 10.1016/j.tree.2008.06.015

Ray, S., Basu, S., Rivera-Vega, L. J., Acevedo, F. E., Louis, J., Felton, G. W., et al. (2016). Lessons from the far end: caterpillar frass-induced defenses in maize, rice, cabbage, and tomato. *J. Chem. Ecol.* 42, 1130–1141. doi: 10.1007/s10886-016-0776-x

Ray, S., Gaffor, I., Acevedo, F. E., Helms, A., Chuang, W. P., Tooker, J., et al. (2015). Maize plants recognize herbivore-associated cues from caterpillar frass. *J. Chem. Ecol.* 41, 781–792. doi: 10.1007/s10886-015-0619-1

Rigby, M. C., Hechinger, R. F., and Stevens, L. (2002). Why should parasite resistance be costly? *Trends Parasitol.* 18, 116–120. doi: 10.1016/s1471-4922(01)02203-6

Rohr, J. R., Swan, A., Raffel, T. R., and Hudson, P. J. (2009). Parasites, infodisruption and the ecology of fear. *Oecologia* 159, 447–454. doi: 10.1007/s00442-008-1208-6

Rusch, A., Valantin-Morison, M., Sarthou, J. P., and Roger-Estrade, J. (2013). Effect of crop management and landscape context on insect pest populations and crop damage. *Agric. Ecosyst. Environ.* 166, 118–125. doi: 10.1016/j.agee.2011.05.004

Ruther, J., Meiners, T., and Steidle, J. L. (2002). Rich in phenomena-lacking in terms. a classification of kairomones. *Chemoecology* 12, 161–167. doi: 10.1007/pl00012664

Sapolsky, R. M., Romero, L. M., and Munck, A. U. (2000). How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Rev.* 21, 55–89. doi: 10.1210/er.21.1.55

Sarabian, C., Curtis, V., and McMullan, R. (2018). Evolution of pathogen and parasite avoidance behaviors. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170256. doi: 10.1098/rstb.2017.0256

Schreier, K. C., and Grindstaff, J. L. (2020). Repeatable behavioral and immune defense strategies against infection are not traded off. *Anim. Behav.* 162, 11–22. doi: 10.1016/j.anbehav.2020.01.008

Selbach, C., and Mouritsen, K. N. (2020). Mussel shutdown: does the fear of trematodes regulate the functioning of filter feeders in coastal ecosystems? *Front. Ecol. Evol.* 8:396.

Sharp, J. G., Garnick, S., Elgar, M. A., and Coulson, G. (2015). Parasite and predator risk assessment: nuanced use of olfactory cues. *Proc. R. Soc. Lond. B Biol. Sci.* 282:20151941. doi: 10.1098/rspb.2015.1941

Sheehan, G., Farrell, G., and Kavanagh, K. (2020). Immune priming: the secret weapon of the insect world. *Virulence* 11, 238–246. doi: 10.1080/21505594.2020.1731137

Sheldon, B. C., and Verhulst, S. (1996). Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* 11, 317–321. doi: 10.1016/0169-5347(96)10039-2

Sheriff, M. J., and Thaler, J. S. (2014). Ecophysiological effects of predation risk; an integration across disciplines. *Oecologia* 176, 607–611. doi: 10.1007/s00442-014-3105-5

Sheriff, M. J., Krebs, C. J., and Boonstra, R. (2009). The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *J. Anim. Ecol.* 78, 1249–1258. doi: 10.1111/j.1365-2656.2009.01552.x

Sheriff, M. J., Orrock, J. L., Ferrari, M. C., Karban, R., Preisser, E. L., Sih, A., et al. (2020a). Proportional fitness loss and the timing of defensive investment: a cohesive framework across animals and plants. *Oecologia* 193, 273–283. doi: 10.1007/s00442-020-04681-1

Sheriff, M. J., Peacor, S. D., Hawlena, D., and Thaker, M. (2020b). Non-consumptive predator effects on prey population size: a dearth of evidence. *J. Anim. Ecol.* 89, 1302–1316. doi: 10.1111/1365-2656.13213

Sih, A., Bolnick, D. I., Luttbeg, B., Orrock, J. L., Peacor, S. D., Pintor, L. M., et al. (2010). Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos* 119, 610–621. doi: 10.1111/j.1600-0706.2009.18039.x

Stephenson, J. F., Perkins, S. E., and Cable, J. (2018). Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies. *J. Anim. Ecol.* 87, 1525–1533. doi: 10.1111/1365-2656.12885

Turlings, T. C., and Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annu. Rev. Entomol.* 63, 433–452. doi: 10.1146/annurev-ento-020117-043507

Underwood, N. (2000). Density dependence in induced plant resistance to herbivore damage: threshold, strength and genetic variation. *Oikos* 89, 295–300. doi: 10.1034/j.1600-0706.2000.890210.x

Vaughan, D. B., Grutter, A. S., Costello, M. J., and Hutson, K. S. (2017). Cleaner fishes and shrimp diversity and a re-evaluation of cleaning symbioses. *Fish Fish.* 18, 698–716. doi: 10.1111/faf.12198

Vilhunen, S. (2006). Repeated antipredator conditioning: a pathway to habituation or to better avoidance? *J. Fish Biol.* 68, 25–43. doi: 10.1111/j.0022-1112.2006.00873.x

Weinstein, S. B., Buck, J. C., and Young, H. S. (2018a). A landscape of disgust. *Science* 359, 1213–1214. doi: 10.1126/science.aas8694

Weinstein, S. B., Moura, C. W., Mendez, J. F., and Lafferty, K. D. (2018b). Fear of feces? tradeoffs between disease risk and foraging drive animal activity around raccoon latrines. *Oikos* 127, 927–934. doi: 10.1111/oik.04866

Werner, E. E., and Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84, 1083–1100. doi: 10.1890/0012-9658(2003)084[1083:arotii]2.0.co;2

Wilson, K., and Cotter, S. C. (2009). "Density-dependent prophylaxis in insects," in *Phenotypic Plasticity of Insects: Mechanisms and Consequences*, eds T. Ananthakrishnan and D. Whitman (Plymouth, UK: Science Pub Inc), 381–420.

Wilson-Rich, N., Spivak, M., Fefferman, N. H., and Starks, P. T. (2009). Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu. Rev. Entomol.* 54, 405–423. doi: 10.1146/annurev.ento.53.103106.093301

Wirsing, A. J., Heithaus, M. R., Brown, J. S., Kotler, B. P., and Schmitz, O. J. (2021). The context dependence of non-consumptive predator effects. *Ecol. Lett.* 24, 113–129.

Wisenden, B. D. (2014). "Chemical cues that indicate risk of predation," in *Fish Pheromones and Related Cues*, eds P. W. Sorensen and B. D. Wisenden (Ames, IA: John Wiley and Sons Inc), 131–148. doi: 10.1002/9781118794739.ch6

Yip, E. C., Tooker, J. F., Mescher, M. C., and De Moraes, C. M. (2019). Costs of plant defense priming: exposure to volatile cues from a specialist herbivore increases short-term growth but reduces rhizome production in tall goldenrod (*Solidago altissima*). *BMC Plant Biol.* 19:209.

Zhukovskaya, M., Yanagawa, A., and Forschler, B. T. (2013). Grooming behavior as a mechanism of insect disease defense. *Insects* 4, 609–630. doi: 10.3390/insects4040609

Züst, T., and Agrawal, A. A. (2017). Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annu. Rev. Plant Biol.* 68, 513–534. doi: 10.1146/annurev-arplant-042916-040856

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GLOSSARY

Allocation costs: victim trade-offs in their allocation of limited resources among growth, reproduction and defense (Cipollini et al., 2003).
Constitutive defense: victim defensive traits already present prior to natural enemy attack (Wirsing et al., 2021).
Consumptive effect: negative effect of one organism on another due to direct energy extraction (Buck et al., 2018).
Direct defense: behavioral, morphological and physiological trait changes exhibited by potential victims to defend against natural enemy attack (Kant et al., 2015).
Ecological costs: induced traits for defense against one natural enemy may cause increased susceptibility to other enemies or abiotic stresses, or reduce attractiveness to mutualists (Cipollini et al., 2003).
Indirect defense: a victim defensive response to a natural enemy that involves a third party, such as to aid in enemy removal (Kant et al., 2015).
Induced defense: victim defenses, both direct and indirect, that are activated in response to heightened enemy risk or actual attack (Wirsing et al., 2021).
Non-consumptive effect: a reduction in victim fitness or population abundance due to costly trait changes in response to perceived risk of consumption (Buck et al., 2018).
Opportunity costs: decreased victim competitive status as a result of allocation to defenses in response to natural enemy risk (Cipollini et al., 2003).
Resistance: defense mechanisms by which victims seek to prevent consumption, or to reduce the development or reproduction of their natural enemies (Råberg et al., 2009).
Risk-induced trait response: enemy risk-induced change in a phenotypically-plastic victim trait, including behavior, morphology, physiology, and life history (Peacor et al., 2020).
Tolerance: defensive trait in victims that can reduce or alleviate reductions in fitness owing to consumption by a natural enemy (Råberg et al., 2009).
Trait-mediated indirect effect: an indirect interaction wherein one species alters the phenotype of another, with the resulting trait changes affecting a third species (Werner and Peacor, 2003).

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 24 September 2021
doi: 10.3389/fevo.2021.659570

Bothersome Flies: How Free-Ranging Horses Reduce Harm While Maintaining Nutrition

Daniel I. Rubenstein[1]* **and Lisa H. Feinstein**[2]

[1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, United States, [2] Camelot Animal Hospital, Belleview, FL, United States

The horses of Shackleford Banks, NC, United States are harassed by many species of biting flies. Apart from being a nuisance, their bites can lead to blood loss and transmit disease. As a result, these horses tend to avoid areas where fly abundances are high. Like other free-ranging horse populations, environmental factors such as low wind speeds and high temperatures increase fly loads per horse. Similarly, coat color matters since darker horses attract more flies than lighter ones, especially on hot sunny days. Many horse populations reduce per capita fly loads by living in large groups or by bunching tightly together. Shackleford horses do so, too, but also use wind speed differences among habitats to modulate fly numbers. By adopting a systematic pattern of moving between habitats such that they only visit a habitat when wind speed is high enough to keep fly harassment to a tolerable level, they can avoid being bitten while continuing to forage. Typically, they begin the day foraging on the salt marshes where fly abundance is inherently low and are lowered further by faint early morning breezes. Later in the morning, horses move to grassy patches (swales) when increasing wind speed reduces fly landings there to levels found on the marshes. Later still, when wind speeds peak, horses begin foraging among the sand dunes. At this point wind speeds are high enough so that horses using any habitat will be minimally harassed by flies, thus enabling them to freely choose where to feed based on which habitat meets particular dietary needs for protein, energy and nutrients on any particular day. Hence, Shackleford horses follow the breeze to solve a challenging dilemma of maintaining a high nutritional plane without succumbing to fly harassment. Other free-ranging horses populations appear to have a more limited "either-or" choice of "bite or be bitten," thus limiting their decision-making options.

Keywords: horses, foraging behavior, avoidance of biting flies, movements, balancing tradeoffs

OPEN ACCESS

Edited by:
Janet Koprivnikar,
Ryerson University, Canada

Reviewed by:
Sarah King,
Colorado State University,
United States
Sara B. Weinstein,
The University of Utah, United States

***Correspondence:**
Daniel I. Rubenstein
dir@princeton.edu

Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: 27 January 2021
Accepted: 27 August 2021
Published: 24 September 2021

Citation:
Rubenstein DI and Feinstein LH
(2021) Bothersome Flies: How
Free-Ranging Horses Reduce Harm
While Maintaining Nutrition.
Front. Ecol. Evol. 9:659570.
doi: 10.3389/fevo.2021.659570

INTRODUCTION

Biting files are not just a nuisance to horses and other large-bodied mammals. When flies alight, they can bite, lead to blood loss, transmit disease, and generally disrupt behavior (Askew, 1971). To make matters worse, flies have evolved finely tuned mechanisms that use high body temperatures and CO_2 levels as well as dark pelage to find and plague their prey (King and Gurnell, 2010). As a result, an evolutionary arms race has developed among pesky flies and horses and their close evolutionary kin. While zebras have evolved stripes as a way of reducing fly detections (Caro et al., 2014; Larison et al., 2015; Caro, 2016), less distinctively marked horses once detected have evolved behaviors that reduce fly nuisance by swishing tails (Mooring and Hart, 1992), or moving to areas where files are less prevalent (Duncan and Vigne, 1979; Zervanos and Keiper, 1979). But behaviors like these are likely to have opportunity costs. Avoiding fly infested areas when they provide high quality forage (Duncan and Vigne, 1979; Zervanos and Keiper, 1979; King and Gurnell, 2010), for example, create dilemmas that are hard to balance. Sometimes areas are avoided for almost entire seasons (Powell et al., 2006).

Many species in the horse family (Equidae) are not without some means for managing these types of tradeoffs without having to move or avoid habitats. As Rubenstein and Hohmann (1989) have shown, horses (*Equus caballus*) can increase the rate at which they swish their tails and shake their manes as fly abundance increases. While investing in comfort behavior may reduce overall time spent feeding or lower bite rate, these reductions are likely to be minimal, especially for those living in large groups where time spent on other time-consuming activities such as vigilance can be reduced by inducing other group members to pick up the slack by lifting their heads. And, as Duncan and Vigne (1979) have shown, banding together in large groups can also directly lower fly nuisance by spreading flies among group mates, thus reducing the per capita number alighting on any particular individual. As Rutberg (1987) notes, however, horses live in closed membership groups, so unlike their close evolutionary kin – the wild asses (*Equus africanus* and *Equus hemionus*) and Grevy's zebras (*Equus grevyi*) – whose societies fission and fuse (Rubenstein, 2011), thus enabling them to easily change group size, horses are unable to quickly change the size of their groups. Instead, when fly numbers are high, horses tend to bunch together to distribute the flies among group members, thus diluting the nuisance for each horse. In fact, once bunched together, rates of tail swishing often increase, further enhancing the per capita benefits of grouping tightly (Mooring and Hart, 1992; Powell et al., 2006). Despite the benefits that such active mutualisms can produce, they are not always shared equally within the group since dominants tend to jostle for places in the center where dilution is most assured and where mutual tail swishing is maximized.

Behavior such as these can indeed lower the impact of biting flies when grazing. But when fly burdens are high, as is often the case during warm spring and summer months when horses are under intense pressure to eat and maintain high body condition in preparation for winter or to rebuild body condition after reproducing, horses in many populations often abandon good grazing areas, seeking refuge on bare ground (Zervanos and Keiper, 1979; Duncan, 2012), elevated sites (King and Gurnell, 2010), or human modified landscapes (Powell et al., 2006). Such chronic reductions in feeding, however, are likely to induce real costs (Mayes and Duncan, 1986). This will be especially true if high quality foraging areas have to be abandoned (King and Gurnell, 2010) for long periods and only visited when fly numbers are low (Berger, 1986; Powell et al., 2006).

Is there anything that horses can do to avoid this challenging choice? Is it possible to reduce the risk of being bitten while still maintaining access to high-quality feeding sites on a regular basis? Insights on how horses could solve this dilemma emerge from how their close kin – zebras – adjust their behavior and activity patterns to simultaneously reduce the risks of being killed by lions without reducing feeding opportunities (Fischhoff et al., 2007). We know from studies on plains zebras (*Equus quagga*), that when a lion makes a kill, or when a zebra detects a lion in a particular habitat, the first response is often for the herd to abandon the area. But by abandoning a chosen grazing site after every such sighting or attack, opportunities to forage there would be lost. To avoid paying such costs, zebras instead alter their "daily round" by varying when they visit essential habits that would enable them to meet their dietary needs. In fact, they vary their visits to these habitats depending on both the expected likelihood of lions occupying particular habitats at particular times of day and by adopting habitat and time specific anti-predator behavior if lions are encountered. Thus, most zebras forage during the day on open grasslands when lions are usually shading in woodlands and they do so by moving deliberately, slowly, and quietly. But during the night or crepuscular periods when lions typically move to the plains to hunt, most zebras move into the woodlands. And when they do, they continue to move deliberately, slowly and quietly presumably letting stripes and leaves co-mingle to provide camouflage (Caro, 2016), thus reducing the risk of being detected and attacked. For those remaining on the open plains, however, their movements change dramatically, becoming more protean, more unpredictable. At night on the plains, zebras move more quickly and turn more frequently and erratically than when grazing there during the daytime (Fischhoff et al., 2007). Like the zebras solving the life-dinner tradeoff (Dawkins and Krebs, 1979), might Shackleford horses adjust their temporal patterns of habitat use to avoid high fly densities without having to forgo feeding in particular regions of the island?

MATERIALS AND METHODS

To answer this question, we observed the free-ranging feral horses of Shackleford Banks, NC, United States during late spring and early summer (June–July) of 1994. These horses have inhabited the island since the mid-1500s and have roamed freely without human interference since the end of the 19th century when people abandoned the island (Rubenstein, 1981). In the mid-1990s the National Park Service began managing the island as a wilderness area and at the time of the study, approximately 220 horses inhabited the island dividing themselves into 20–25 family groups, each consisting of one or more breeding stallions, females and their young, or all male bachelor groups. Shackleford Banks is a barrier island that is 15 km long and 2 km wide at its widest point and consists of five major habitat types – beach, dunes, grassy swales, forests, and salt marshes–that generally range linearly along the long-axis of the island with the salt marshes and forests lying along the sound, with the dunes and swales occupying the middle of the island between beach and sound (Rubenstein, 1981). Only the latter four habitats are used for grazing.

During daily censuses, we gathered six types of data. First, we observed where and when horses grazed in each habitat. Since the island is open and the horses spend virtually all their time on the salt marshes, swales, and sand dunes, they are easy to spot when traversing the island on foot. Foot surveys of each habitat at different times of day were used to find horses and ensure that their habitat choices spanned all daylight hours. Between a third to half of the island can be walked per day while stopping and undertaking hour-long scan samples, so each group was followed for long periods at least 3–4 times per week. If a group was followed continuously for 1–2 h, each habitat it occupied during that interval was recorded. If a group was watched for less than an hour, it was typically sighted and re-sighted multiple times per day in a variety of habitats and tens, if not hundreds, of times during the summer. Each time a group was opportunistically spotted, the habitat it was occupying was noted. Accumulating many sightings of each group helped ensure that habitat occupancy was representative of each group's preferences. In addition to recording habitat occupancy, time of day when a group entered or left a particular habitat was recorded and used to compute proportionate time of occupancy for each habitat. This allowed us to determine habitat selectivity, or preferences, using Ivlev's Electivity Index (Ivlev, 1975) which compares the proportion of the island covered by each habitat to the proportion of time horses were recorded in each habitat.

$$Electivity = (P_{HabitatUse} - P_{HabitatAvailable})/(P_{HabitatUse} + P_{HabitatAvailable}),$$

where $P_{HabitatUse}$ is the proportion of time horses were sighted using a particular habitat and $P_{HabitatAvailable}$ is the proportion of the island consisting of that habitat.

Second, we recorded behavioral time budgets using scan samples (Altmann, 1974) of each individual's actions (grazing, standing, or walking) at 5-min intervals for a period of 60 min throughout the day from 7.00 to 18.00 h. Since the horses are habituated to island visitors, behavioral data and fly counts were routinely gathered at distances of 5–10 m. A total of 13 groups, ranging in size from 3 to 15 individuals, were regularly sighted and re-sighted over the summer generating 146 h of scan samples. On average, each group was followed for approximately 11 h. Short opportunistic sightings were also used to record habitat use and associated environmental states at that time and place along with observations of additional social interactions. Third, we gathered data on available vegetation in each habitat to assess diet quality and quantity. Where horses were seen grazing, we directly measured vegetation abundance at that site by walking 25 m transects using "pin drops" (Crawford et al., 2019) to count the number of leaves, stems or seed heads of each grass species touching a 1 mm wide welding rod. Each habitat was sampled where the horses were seen grazing at least 10 times during the spring and summer. By measuring habitat electivity and species frequency, we could estimate the relative abundance of the various vegetation species available to horses as they moved among habitats [salt marsh, swale (grassy patches), dunes, and forest edges] on their "daily rounds." For one, or at most two, of the numerically dominant species making up >10% of the area of a particular habitat, the crude protein, digestible energy, ash free detergent fiber (ADF) and key micronutrients nutrients (Ca, K, P, Cu, and Zn) were determined so that the relative importance of each habitat in helping meet daily dietary needs could be compared (**Supplementary Table 1**). The nutrient content and % crude protein of each of these key species was extracted from Balbo (1985) and Pratt-Phillips et al. (2011) and various USDA websites.

Fourth, during gaps between scan sampling intervals we also counted from head and to tail the number of biting flies of four species– Green headed horseflies (*Tabanus nigrovittatus*) and *Tabanus lineola*, deer flies (*Chrysops fuliginosus*) and (*Chrysops atlanticus*), as well as stable flies (*Stomoxys calcitrans*) (Rubenstein and Hohmann, 1989) – alighting on or hovering over (within 5 cm of the body), horses at different times of day. These fly species were all large enough to count individually from 5 m away or by binoculars when more distant, but they were not identifiable to species at these distances. They were also the species that visibly annoyed the horses, inducing head shakes, muscle twitches or tail swishes. Since neither non-biting flies nor mosquitoes evoked these behaviors, neither were recorded. We also measured general weather conditions by recording daily temperature (average and maximum) and sky cover (clear, overcast, or partly overcast).

Fifth, whenever males interacted aggressively, which ranged from displays, to calls, to sniffs to physical contacts, we recorded the identity of the contestants and recorded who won and who lost. Fights typically terminated when losers "head bobbed" and walked away. These pairwise won-loss outcomes populated the cells of a winner-loser matrix from which pairwise dominance was determined. By moving the rows up or down to minimize the number of values in the matrix's bottom triangle a linear rank ordering was determined. The top 50% were designated as dominants while the bottom 50% were designated as subordinates (Rubenstein, 1994). This hierarchy helped determine the role that rank could play in shaping movements among habitats on the island.

And sixth, wind speed in each habitat was recorded with and without horses present using a handheld wind gauge to compute average wind speed (km/h) in each of the habitats throughout the day to determine if and when, wind speed might enable horses to enter particular habitats since biting fly numbers typically decrease as wind speed increases (Rubenstein and Hohmann, 1989; King and Gurnell, 2010).

Statistical analyses involved standard analyses of variance, *t*-tests and regressions since the data were normally distributed. Data consisting of counts were analyzed via Chi-squared contingency tables. For clarity, the figures generally present the central tendencies of the main effects or their interactions. The full statistical analyses are based on hundreds of measurements of individuals or habitat features and are presented along with significance levels in the text or figure legends.

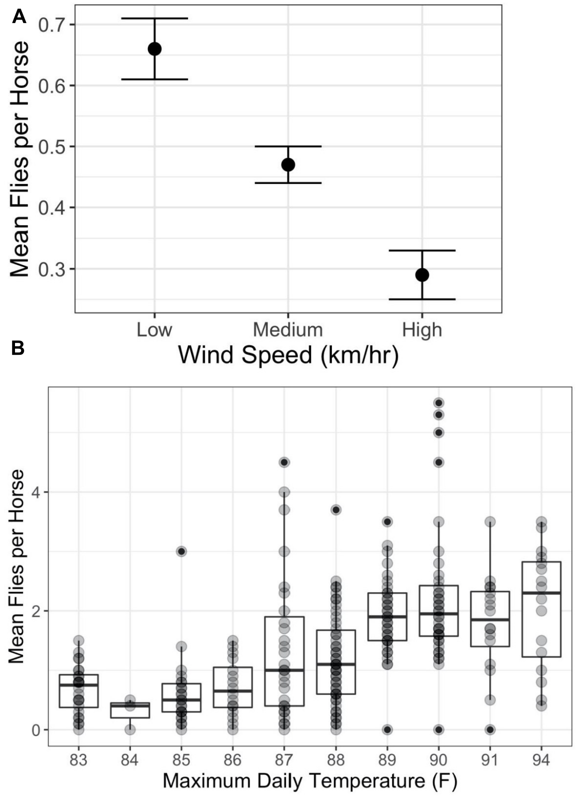

FIGURE 1 | Relationship between per capita fly numbers and environmental factors. **(A)** Impact of wind speed on per capita numbers (mean and standard error) and wind speed category (Low = 0–5 km/h; Medium = 6–15 km/h; and High >15 km/h) ($F_{2,750} = 12.2$; $p < 0.0001$). **(B)** Impact of daily maximum temperatures on per capita numbers (mean and standard error) ($F_{9,229} = 11.78$; $p < 0.0001$; $R^2 = 0.32$). Correlations between maximum temperature and mean daily fly numbers are positive and significant from 83°F to 89°F ($F_{1,169} = 42.17$; $p < 0.0001$; $R^2 = 0.20$), but level off showing no statistically significant correlation above 89°F ($F_{1,99} = 0.01$; $p < 1$; $R^2 = 0$). Darker points represent multiple occurrences.

RESULTS

Are Shackleford Horses Harassed by Flies?

The answer is "Yes" with the degree of harassment depending on environmental conditions, phenotype, and behavior. Overall, on Shackleford Banks per capita fly loads decrease with increasing wind speed (**Figure 1A**) ($F_{2,750} = 12.2$; $p < 0.0001$) and increase with increasing temperature (**Figure 1B**) ($F_{9,229} = 11.78$; $p < 0.0001$). In addition, coat color makes a difference. Generally, darker colored horses attract more flies per capita than lighter colored horses, especially on sunny days (**Figure 2**) ($F_{9,229} = 11.78$; $p < 0.0005$).

Social factors also play significant roles in determining per capita fly loads. As in other free-ranging populations of feral (Duncan and Vigne, 1979; Rutberg, 1987) and wild (King and Gurnell, 2010) horses, those on Shackleford Banks living in larger groups are bothered by fewer flies than those living in smaller groups (**Figure 3A**) ($F_{2,935} = 64.9$; $p < 0.0001$). Even though the absolute number of flies landing or hovering close to the horses are inherently low (fewer than five at most instances), their determined attempts to alight and bite can be very disruptive. Accordingly, when disturbed, Shackleford horses bunch tightly together at any group size to further reduce per capita fly loads (**Figure 3B**) ($F_{1,761} = 37.61$; $p < 0.0001$).

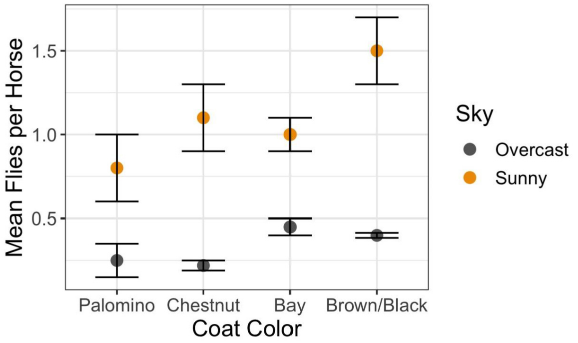

FIGURE 2 | Relationship between the morphological trait of coat color under different sky conditions on per capita fly loads (mean and standard error: Interaction Sky Condition × Coat Color = $F_{3,622} = 5.94$; $p < 0.0005$). Coat colors range from the light golden Palominos with cream-colored manes to reddish-brown bodies and manes of Chestnuts, and to the brown bodies of the Bays highlighted with blackish manes, to those with solid dark brown or black bodies with matching manes.

Does Fly Harassment Create Non-disease Foraging Costs?

Clearly, Shackleford horses are disturbed by biting flies much like other free-ranging horse populations. In those studies, when fly numbers are high, horses seek refuge in habitats where fly numbers are low. But in those studies, taking refuge is not without costs. In Mongolia, Przewalski horses seek high ground where forage has been denuded, only returning to preferred feeding habitats when fly numbers decline in those habitats (King and Gurnell, 2010). The same occurs in the Camargue (Duncan and Vigne, 1979) where horses seek bare ground to escape harassment. And even on Assateague Island, a neighboring barrier island north of Shackleford Banks, the horses there move out into the water to reduce harassment (Rutberg, 1987), or to areas with human built structures during seasons when fly numbers are high (Powell et al., 2006). Each of these strategies, however, results in lost feeding opportunities. Do the Shackleford horses suffer the same fate? The answer is "No."

How Then, Do Shackleford Horses Manage to Avoid Incurring Foraging Costs Without Being Harassed by Flies?

To show how they solve this challenging dilemma, we first need to characterize the quality of foraging opportunities offered by each habitat on Shackleford Banks. Using our vegetation data, we determined: (1) the identity and quality of the dominant grasses in each habitat; (2) the degree to which horses use each of the four

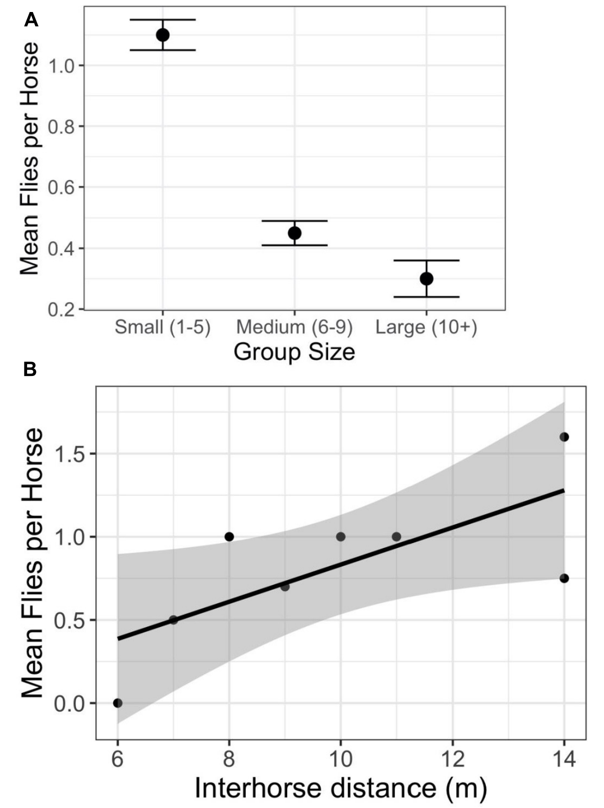

FIGURE 3 | Relationship between per capita fly numbers and social factors. **(A)** The relationship between group size and the average number of flies hovering over, or landing on, individual horses ($F_{2,935} = 64.9$; $p < 0.0001$). **(B)** The relationship between the average number of flies hovering over, or landing on, individual horses as a function of the average distance between two horses within groups ($y = 0.53 + 0.12^{*}X$; $F_{1,761} = 37.61$; $p < 0.0001$).

habitats for grazing; (3) the relative nutritive value horses could derive by feeding in each habitat; and finally, (4) the degree to which habitat use correlates with fly abundance, specifically the degree to which the underuse of one or more habitats correlates with high fly burdens. **Supplementary Table 1** provides the data necessary to address the first three points.

First, each habitat is dominated by at least one unique grass species (those whose % ground cover exceeded 10%; **Supplementary Table 1**). Second, horses do not exploit the habitats equally, nor do they even frequent them in proportion to their relative abundance. As Ivlev's Electivity Index in **Supplementary Table 1** shows, horses on average elect (positive values >0.1) to feed mostly in the relatively less common marsh (+0.44) and swale (+0.49) habitats, while mostly avoiding (negative values <−0.1) the relatively most common dune (−0.77) and forest habitats (−0.89). Third, in all but the forest habitat, one or more of these numerically dominant species provides a high level of crude protein (>20%). Thus, at least in terms of providing essential nitrogen, the habitats are all generally good, although the swale habitat provides more nitrogen rich plant species for grazing than any of the other habitats. Yet in terms of digestible energy [low levels of Acid Detergent Fiber (ADF)], the habitats differ markedly with the swales and dunes offering the most digestible species–pennywort (*Hydrocotyle bonariensis*)–and the forest edges offering highly digestible panic grasses.

The largest differences among habitats, however, emerges from the micronutrients that the habitat specific grasses have to offer. The salt marsh and swale habitats are highest in % phosphorous because *Spartina* sp. are abundant, whereas the dunes and the swales are highest in % calcium because pennywort (*Hydrocotyle bonariensis*) is common. High levels of copper can be found in all but the dunes and for acquiring zinc, foraging in either the forest or swales is necessary. Overall, the habitat that comes closest to offering most of what horses need (NRC, 2007) is the swale habitat, but its most abundant grass, *Spartina patens*, is not easy to digest and provides relatively low levels of digestible energy. Thus, grazing in each habitat offers something nutritively unique since no habitat provides vegetation that is high in energy, protein and all essential micronutrients. In fact, according to the National Resource Council (NRC, 2007), virtually all vegetation on Shackleford during spring and summer provides a micronutrient deficient diet (Pratt-Phillips et al., 2011). To minimize these deficiencies, horses should choose habitats where forage species offering the highest nutrient levels are most abundant. To do this, horses should spend time in the dunes to maximize phosphorus intake, time in the swales to maximize copper intake, time in the marshes to maximize calcium intake and time in both the forest and swales to maximize zinc intake. Clearly, visiting all four habitats is essential if the horses on Shackleford Banks are going to maintain as high a level of bodily condition as is possible.

Is Differential Habitat Use Related to Habitat Differences in Fly Abundance?

The answer is "Yes." When in preferred habitats – salt marsh and grassy swale – horses are harassed by flies on a per capita basis significantly less than when in less preferred habitats – dunes and forests (**Figure 4**) ($F_{3,663} = 25.3$; $p < 0.0001$). Why might this be so? Flies have a difficult time coping with strong winds, as illustrated in **Figure 1A**; they find it more and more difficult to detect and land on horses when wind speeds are high. However, on average, wind speeds vary by less than 2.5 km/h among marsh (13.3 km/h), swale (12.4 km/h), and dune (14.9 km/h) habitats. Only the forests show significantly lower average wind speeds (1.5 km/h). If horses were to access habitats solely with respect to the impact that wind speed has on fly landings, then horses should avoid the forests and favor the salt marsh, swale, and dune habitats more or less equally. But the horses do not do this (**Supplementary Table 1**). Forests are indeed the least preferred habitat ($E = -0.89$) and are used the least (**Figure 5**). But the dune habitat, despite being windswept for much of the day, is not favored either ($E = -0.77$), at least until the end of the day. And given that horses do occasionally enter forests, but only when wind speeds are way above the habitat's average (∼ 20 km/h), something more subtle appears to be operating.

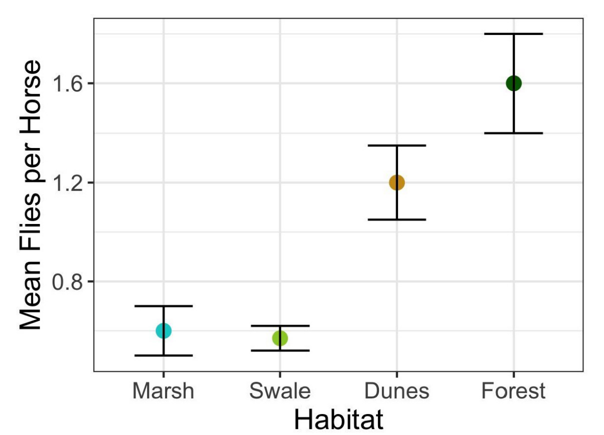

FIGURE 4 | Mean and standard error of average fly landings per horse during the summer for the four major Shackleford Banks habitats ($F_{3,663} = 25.3$; $p < 0.0001$).

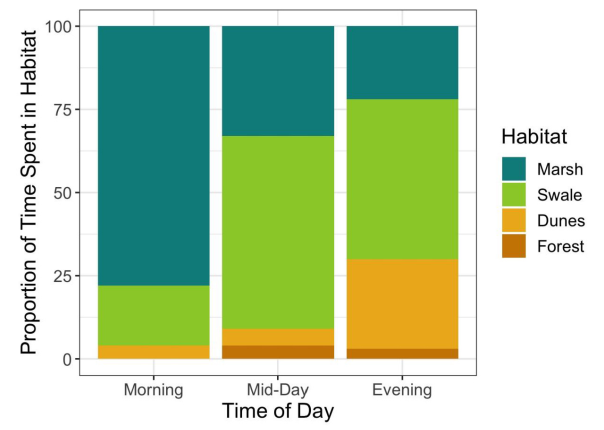

FIGURE 5 | Relationship between the proportion of horses sighted in each habitat during different periods of the day. The Shannon Diversity index increases as the day progresses, showing that horses are using habitats more evenly as wind speed increases (Morning = 0.27; Mid-Day = 0.42; and Evening = 0.50).

How Might Horses Use Habitat Specific Wind Speeds to Limit Fly Harassment While Enhancing Overall Foraging Success?

As **Figure 6** shows, averages do not reveal the full story. Wind speed increases everywhere throughout the day, but differently in each habitat (Interaction between habitat × time of day: $F_{6,710} = 47.5$; $p < 0.0001$). Early in the morning wind speeds are generally low in all habitats compared to later in the day. Yet in the early morning, wind speeds are markedly higher in the marsh than in any other habitat. And although they increase in all habitats from early morning onward, they tend to level off in the marshes, swales, and forest by mid-day. Only in the dunes do they continue to rise well into the evening.

If horses could adjust the timing of when they enter and leave habitats to match the times when fly abundances in those habitats are low, then perhaps they could reduce fly harassment without having to forgo the unique and beneficial foraging opportunities that each habitat has to offer. To evaluate this proposition, some simple calculations derived from **Figure 7** are instructive. **Figure 7** illustrates the relationship between wind speed and the average number of fly landings per horse when in each habitat. Increases in wind speed decrease per capita fly landings non-linearly, converging to approximately 0.33 flies per horse in all habitats at high wind speeds, those greater than 25 km/h. But at low wind speeds harassment levels vary by habitat, being highest in the forest, then declining first in the dunes, then the swales and finally the marshes, respectively. Hypothetically, if horses were trying to maintain a steady state of "hosting" only one fly at all times, then they could do so by entering the salt marshes when wind speed were greater than ∼1 km/h and swales when they reached ∼3 km/h. But to enter dune or forest habitats while keeping fly landings to one per horse, horses could only do so if wind speeds exceeded 10 km/h and 20 km/h, respectively. Since such high wind speeds are almost never reached daily in the forests, it is not surprising that horses rarely enter them (**Figure 5**). When they do, it is either during storms when wind gusts are strong and frequent, or after mid-day and into the evening when wind speeds in general, are at their highest levels. For the other three habitats, Shackleford horses appear to utilize habitats when the critical thresholds depicted in **Figure 7** are crossed. In the early morning, horses spend more than 75% of their time in the marshes when wind speeds are low. And although they are well below what the average will be in the salt marsh later in the day (13.3 km/h), they are well above the level necessary to minimize fly landings. As wind speeds increase by mid-morning (**Figure 6**), horses start spending most of their time in the swales (∼55%). Still, they spend ∼30% of their time in the marshes since continuing high wind speeds there maintain fly loads at levels found on swales. From mid-day through evening, when the highest wind speeds occur in the dunes, fly loads in the dunes finally decline to levels matching those in the marshes and swales. At this point, horses are essentially free to forage in the dunes – or any of the habitats apart from the forests– without incurring any increased harm from biting flies. And as **Figure 5** shows, by evening, the horses spend ∼25% of their time in the dune habitat. In general, habitats are used more evenly as wind speed increases throughout the day (Shannon Diversity Indices by time of day: morning = 0.27; mid-day = 0.42; and evening = 0.50).

Do All Horse Groups Use Wind Speed to Move Among Habitats in the Same Way?

The answer is "No." In the other horse populations described above, horses in different sized groups suffer different degrees of fly harassment and even adopt behaviors such as clustering tightly and increasing mutual tail swishing to try and ameliorate high fly loads (see **Figures 3A,B**; Duncan and Vigne, 1979; Mooring and Hart, 1992; Powell et al., 2006). Typically, horse groups are of closed membership, containing one male and many

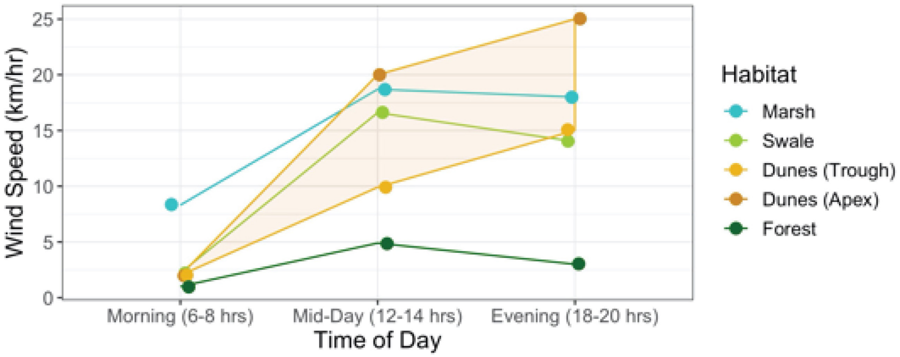

FIGURE 6 | Relationship between wind speeds per habitat at varying times of the day (morning, mid-day, and evening) (Interaction Habitat × Time of Day: $F_{6,710} = 47.5$; $p < 0.0001$). The shaded band highlights the areas in the dunes between the apex and trough where wind speeds are at their highest and lowest, respectively.

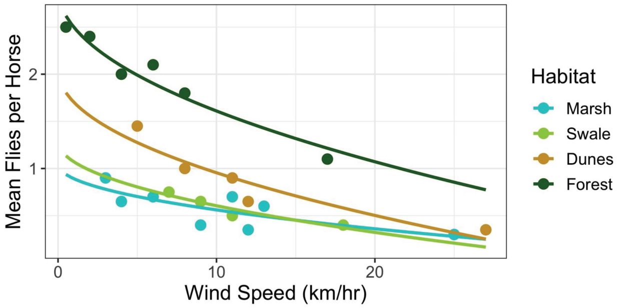

FIGURE 7 | Relationship between wind speeds (km/h) and the average number of fly landings per horse when in each habitat [Marsh: $y = 1.15 - 0.63*\log(x)$, $R^2 = 0.72$; Swale: $y = 1.45 - 0.84*\log(x)$, $R^2 = 0.96$; Dunes: $y = 1.46 - 0.84*\log(x)$, $R^2 = 0.94$; Forest: $y = 2.47 - 0.85*\log(x)$, $R^2 = 0.79$]. Note, that fly landings decline non-linearly as wind speed (km/h) increases in all habitats. Apart from the forest habitat, however, the decline approaches an asymptote of around 0.33 flies per horse at 25 km/h.

females along with their associated young. During the spring and summer breeding seasons, harem stallions fight among themselves to prevent being cuckolded and to secure mating opportunities with females not of their own groups (Rubenstein, 1986). Such contests establish a strong dominance hierarchy among males (Rubenstein, 1994). If the consorted female favors such a dominant male or one rising quickly in rank, she typically joins his group because over time dominant stallions are best able to increase female foraging success by keeping cuckolding males away (Rubenstein, 1986). As a result, the groups of favored, dominant males grow.

Because dominance enables males of high rank to move freely about the island, they should be more able than subordinates to occupy habitats of their choosing when they want. With their enhanced fighting ability, their groups should be the ones most free to use wind speed to access particular habitats at optimum times of day. And this is what occurs on Shackleford Banks. The relatively rare salt marsh and grassy swale habitats should be preferred by competitive dominants during early morning when wind speeds are low (<10 km/h) if foraging gains are to be maximized while fly loads are to be minimized (**Figure 7**). Of the horses sighted during the early morning hours, 100% of horse groups using salt marsh habitats are associated with dominant males–those in the top half of the hierarchy (**Figure 8**). For groups associating with low-ranking males, however, only 40% are sighted in these habitats. 60% of the sightings of groups with subordinate males are seen in the dunes or forest where fly loads at these times of day are much higher than in the marshes or swales (Fisher Exact: $p < 0.0001$). When wind speeds exceed 10 km/h, however, dominance-dependent habitat use patterns essentially disappear. By the time wind speeds reach high levels, fly landings in most habitats are reduced to very few. At this point, dominant male groups forage in the marsh and swales 88% of the time which does not differ significantly from use patterns displayed by subordinate male groups which are seen grazing in them 81% of the time. Similarly, while groups associated with subordinate males were found in the dune and forest habitats 19% of the time when wind speeds exceeded 10 km/h, those with dominant males were seen there 12% of the time (Fisher Exact: $p = 0.523$). Thus, male status and its impact on group size, influences the ability of their females and their offspring to use habitats differently during the day to modulate fly harassment thus maximizing foraging rate. While dominant males and their larger groups are free to enter habitats at optimal times, subordinate males and their smaller groups are not.

Thus, Shackleford horses solve the challenging dilemma by using habitats at different times of day to modulate fly loads. In general, this leads to a very predictable "daily round" (**Figure 9**). Horses begin the day in the salt marsh where fly loads are normally low and even a mild breeze will keep them low enough so that fly modulating behavior does not impede efficient foraging. Then many groups move to the swale where modest increases in wind speed depress fly activity to levels no different from those found in the marsh. There they remain until mid-day when stronger winds sweep across the island, finally enabling the horses to forage in the dunes. By adopting this predictable pattern of movement, horses can access their food and be free of fly harassment to eat it.

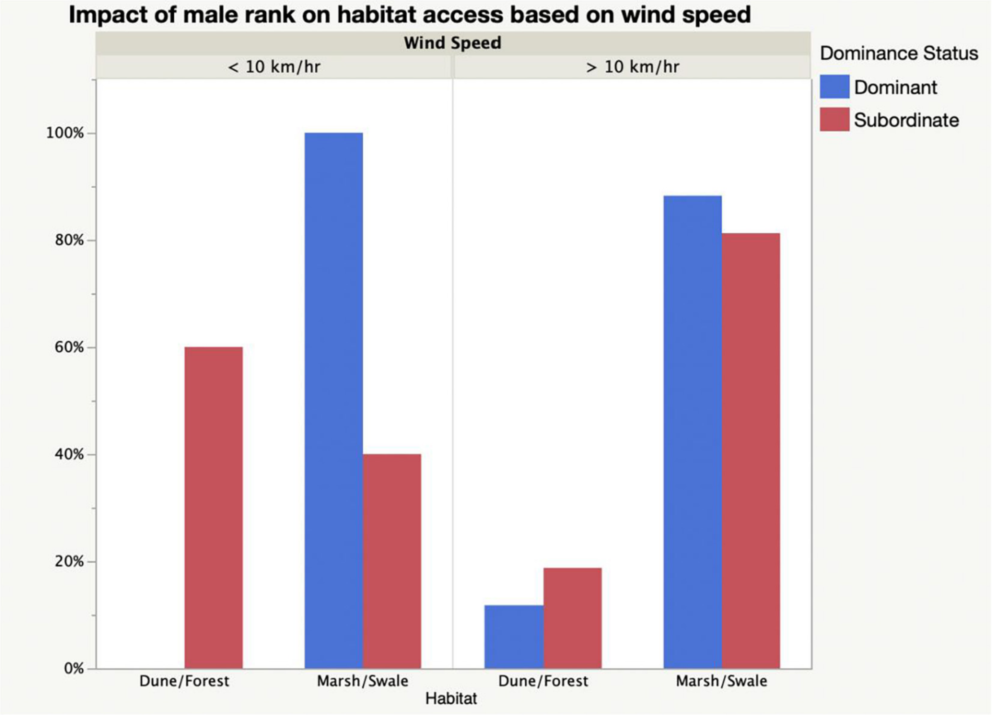

FIGURE 8 | Relationship between male dominance status and the use of relatively rare habitats (marsh and swale) and relatively abundant habitats (dune and forest) as a function of low (<10 km/h) and high (>10 km/h) wind speeds. At low wind speeds groups associated with dominant males exclusively used marsh and swale habitats (Fisher exact: $p < 0.0001$), but at higher wind speeds habitat access was independent of male status (Fisher exact: $p = 0.5229$).

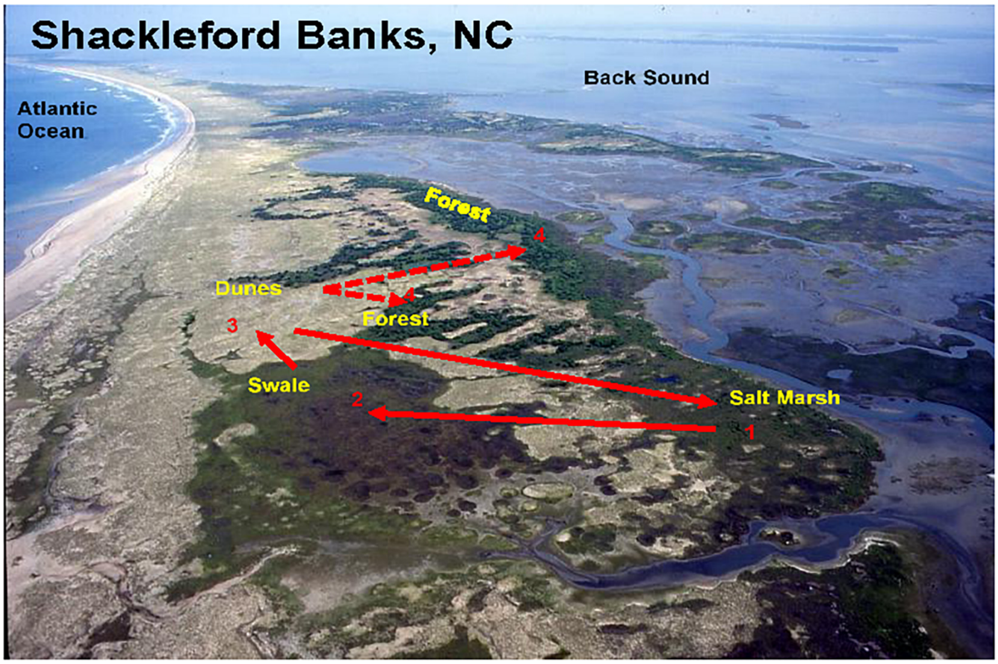

FIGURE 9 | Aerial view of the island showing that the major habitat types are distributed along the long axis of the island. The numbers connected by arrows depicts a typical "daily round" – the numbers depict the order in which the horses move among habitats: from the salt marshes (1) to the swales (2), to the dunes (3), and the forests (4). Solid lines show the normal movement order among habitats on days when wind speeds range from 1 to 15 km/h. Dotted lines represent occasional movements to forest habitats when wind speeds exceed 20 km/h.

DISCUSSION

The calculations presented above concerning how horses can adjust their behavior to have their forage and eat it without harassment from flies illustrate three key points about the relationship between biting flies and how they affect the behavior of free-ranging Shackleford horses with respect to habitat use. The first point is that the horses inhabiting this barrier island are affected by flies in ways very similar to those of other horses living in different locales and occupying different landscapes. Like wild horses in Hustai National Park, Mongolia King and Gurnell (2010), or on Assateague Island National Park in the United States (Zervanos and Keiper, 1979; Rutberg, 1987; Powell et al., 2006) or in the Camargue region of southern France (Duncan and Vigne, 1979) individuals with dark coats or living in large groups, especially experiencing high temperatures or low wind speeds, are pestered by high numbers of biting flies. And just as those horses attempt to reduce such harassment by increasing tail swishing and bunching together to share the burden and reduce per capital fly loads, so do the Shackleford horses.

The second point is that although the Shackleford horses change habitats as fly numbers increase just as do the horses on Assateague or in the Camargue and Mongolia, the horses on Shackleford do so in more nuanced ways. In the other populations, when temperatures reach their peak, horses abandon prime grazing areas, seeking refuges where they rest bunched together swishing their tails. Within seasons, in both the Mongolia and Camargue, horses leave valleys to climb to high ground or move to bare areas where fly numbers are lower. The same occurs between seasons on Assateague Island. There, horses rarely frequent the scrub habitat during the summer, instead spending a disproportionate amount of time in the dunes and in human modified habitats. And while wind speed directly mediates fly numbers in the Camargue, in Mongolia and on Assateague, wind speed modulates tail swishing which indirectly likely reduces fly harassment. Thus, in both populations a "daily" or even a "seasonal" round is established, but it is a simple one, involving either back and forth movements on a daily basis between heavily infested foraging areas and fly free refuges, or by avoiding habitats during seasons when they are heavily infested with flies, returning to them in future seasons after fly loads decline.

Shackleford horses also adopt a "daily round," but it is more nuanced and diverse, fostering behavioral flexibility. By adopting a "daily round" that takes advantage of predictable time-of-day, habitat specific, wind speed changes, Shackleford horses avoid an "either-or" "eat or be bitten" situation. Their daily round is structured around generally predictable graded changes in wind speed, enabling sustained grazing throughout the day by letting the wind modulate fly numbers. Since increases in wind speed occur predictably at different times of day in different habitats, some horses – those associating with dominant males–can time their movements to maintain almost constant fly loads, thus potentiating optimal patterns of daily access to three of the four habitats. The "daily round" begins with morning grazing on salt marshes because only on such marshes can horses consume vegetation rich in protein and calcium and of moderate energy value (*Spartina alterniflora*) without being harassed by flies. On the marsh, even the slightest breeze reduces inherently low fly numbers to levels that make grazing essentially hassle free. As the day progresses, wind speeds increase everywhere on the island, so horses could remain in the marshes to graze because fly landings would continue to decline. Or they could take advantage of higher wind speeds and move to the swales as the morning progresses. Here they would also experience low fly numbers matching levels horses would be experiencing on the marshes. But for those that can temporarily defend the small and patchy swales – again, groups associating with dominant males – they are able to forage on a mixture of foods (*Spartina patens* and *Hydrocotyle bonariensis*) that meet many of the horses' essential nutrient needs. After mid-day and into the early evening wind speeds peak. And even though wind speeds are lower in dune valleys than on dune apices, by the time they reach 10 km/h fly landings on horses using the dunes drop on average to levels matching those for horses using the swales and marshes (**Figure 7**). Thus, by evening all horses are free to graze in the dunes without suffering from increasing fly burdens. In fact, by the end of the day, if winds are blowing at 10 km/h or greater, all habitats, apart from the forest, are available for grazing because per capita fly levels are uniformly low. And because the dune habitat is the island's most common habitat, all horse groups irrespective of the dominance rank of their males can easily gain access.

Because forests are only accessible when wind speeds are very high, they will be off limits on most days and thus do not factor into the "daily round." But when stormy conditions arise, fly levels drop and the forest then can be, and is, utilized. While forests provide forage high in copper and especially zinc, they may also be sought out for protection when winds become extremely strong. Further still, while forest habitats are often used by horses in other populations to shade on extremely hot days despite high fly numbers King and Gurnell (2010), Shackleford horses do not seek shade even on the hottest days. Again, on Shackleford Banks increases in wind speed throughout the day mirror increases in temperature, apparently preventing overheating while at the same time reducing fly landings. In addition, since the wind is often on shore from the ocean late in the day, walking on the beach on extremely hot days as horses move between habitats can further reduce both fly numbers and heat loads. **Figure 9** shows an idealized, yet typical, round of daily movements of a typical horse family or bachelor group. The solid arrows connect the sequence of moves and show the movement trajectory among the three most commonly used foraging habitats. The dashed line depicts excursions into the forest when wind speeds become extremely high.

The third point is that habitat entry decisions arising using wind speed to modulate fly loads represents a novel example of an "Ideal Free Distribution" (Fretwell, 2020). Typically, animals adopting an "Ideal Free Distribution," respond directly to habitat adjusted payoffs associated with seeking "bottom-up" resources, usually food or mates. Milinski's (1987) stickleback fish distributed themselves in aquaria "freely" (without any aggressive interference) such that the fish distributed themselves at each feeding station to equalize per capita rewards. On grasslands, if hypothetical groups of horses distributed themselves in accordance with an "Ideal Free Distribution," they would initially avoid habitats where feeding rates were lower than those of the habitats they were occupying. But as food levels declined and feeding rates increased to match those of previously avoided habitats, horses would begin utilizing those habitats, too. What is striking about the Shackleford horses, is that they too, mostly appear to be moving between habitats in an "Ideal Free" fashion. For those that can, they appear to be doing so by equalizing a "top-down" ecological force–fly harassment levels – rather than equalizing a "bottom-up" resource level force. Thus, by following an "Ideal Free" movement rule, most horses – at least those associating with dominant males – can access the unique high quality foraging opportunities that each habitat has to offer. And this benefit provides another selective force enabling dominant males to benefit from living in larger groups. And for the females living in the large groups of dominant males, not only can they use wind speed to move freely among habitats, the large size of the groups also enables them to dilute whatever level of fly harassment they experience by sharing them with the group mates (see **Figure 1A**).

In general, most groups tend to follow a "daily round" moving from salt marshes to swales to dunes because increases in habitat specific wind speeds follow the same sequence (**Figure 6**). Only horses associating with subordinate males are constrained from moving freely among habitats in ways that solve the challenging choice of optimizing foraging gains while reducing fly loads. But this constraint only occurs during early morning. If it is a windy day (speeds more than 10 km/h), by the end of the day, even groups associated with subordinate males are free to move among habitats to satisfy dietary needs because average wind speeds remain high, thus reducing, and equalizing fly landings in the three most used habitats (**Figure 7**). This freedom to choose among habitats at the end of a windy day should help individuals to make up for any nutritional deficiencies accrued throughout the day without being constrained by biting flies. This flexibility of habitat choice helps explain why habitats are used more evenly at the end of the day and shows how Shackleford horses solve the challenging dilemma of being able to eat what they need without excessively being bitten in the process of doing so.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Princeton University IACUC.

AUTHOR CONTRIBUTIONS

DR and LF designed the investigation and gathered and analyzed data. DR wrote the manuscript. LF contributed to edits. Both authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Princeton University and an NSF Presidential Young Investigator Award.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.659570/full#supplementary-material

REFERENCES

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour* 49, 227–266. doi: 10.1163/156853974x00534

Askew, R. R. (1971). *Parasitic insects. Parasitic insects*. London: Heinemann Educational Books, 316.

Balbo, J. (1985). *Vegetation analysis and implications for habitat selection and territoriality in feral horses (Equus caballus)*. Unpublished Thesis. Princeton, NJ: Princeton University.

Berger, J. (1986). *Wild horses of the Great Basin: Social Competition and Population Size*. Chicago, IL: University of Chicago Press.

Caro, T. (2016). *Zebra stripes*. Chicago: University of Chicago Press.

Caro, T., Izzo, A., Reiner, R. C., Walker, H., and Stankowich, T. (2014). The function of zebra stripes. *Nat. Commun.* 5, 1–10.

Crawford, C. L., Volenec, Z. M., Sisanya, M., Kibet, R., and Rubenstein, D. I. (2019). Behavioral and ecological implications of bunched, rotational cattle grazing in East African Savanna ecosystem. *Rangeland Ecol. Manag.* 72, 204–209. doi: 10.1016/j.rama.2018.07.016

Dawkins, R., and Krebs, J. R. (1979). Arms races between and within species. *Proc. R. Soc. Lond. B. Biol. Sci.* 205, 489–511. doi: 10.1098/rspb.1979.0081

Duncan, P. (2012). *Horses and grasses: the nutritional ecology of equids and their impact on the Camargue*, Vol. 87. Berlin: Springer Science & Business Media.

Duncan, P., and Vigne, N. (1979). The effect of group size in horses on the rate of attacks by blood-sucking flies. *Anim. Behav.* 27, 623–625. doi: 10.1016/0003-3472(79)90201-x

Fischhoff, I., Sundaresan, S., Cordingley, J., and Rubenstein, D. I. (2007). Habitat use and movements of plains zebra (*Equus burchelli*) in response to predation danger from lions. *Behav. Ecol.* 18, 725–729. doi: 10.1093/beheco/arm036

Fretwell, S. D. (2020). *Populations in a Seasonal Environment. (MPB-5)*, Vol. 106. Princeton,NJ: Princeton University Press.

Ivlev, V. S. (1975). *Experimental ecology of the feeding of fishes*. New Haven, CT: Yale University Press.

King, S. R., and Gurnell, J. (2010). Effects of fly disturbance on the behaviour of a population of reintroduced Przewalski horses (Equus ferus przewalskii) in Mongolia. *Appl. Anim. Behav. Sci.* 125, 22–29. doi: 10.1016/j.applanim.2010.03.006

Larison, B., Harrigan, R. J., Thomassen, H. A., Rubenstein, D. I., Chan-Golston, A. M., Li, E., et al. (2015). How the zebra got its stripes: a problem with too many solutions. *R. Soc. Open Sci.* 2:140452. doi: 10.1098/rsos.140452

Mayes, E., and Duncan, P. (1986). Temporal patterns of feeding behaviour in free-ranging horses. *Behaviour* 96, 105–129. doi: 10.1163/156853986x00243

Milinski, M. (1987). "Competition for non-depleting resources: The ideal free distribution in sticklebacks," in *Foraging behavior*, eds A. C. Kamil, J. R. Krebs, and H. R. Pulliam (Boston, MA: Springer), 363–388. doi: 10.1007/978-1-4613-1839-2_11

Mooring, M. S., and Hart, B. L. (1992). Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. *Behaviour* 123, 173–193. doi: 10.1163/156853992x00011

NRC. 2007. *The Nutrient Requirements of Horses, 6th revised edn*. Washington, DC: National Academy Press.

Powell, D. M., Danze, D. E., and Gwinn, M. A. (2006). Predictors of biting fly harassment and its impact on habitat use by feral horses (Equus caballus) on a barrier island. *J. Ethol.* 24, 147–154. doi: 10.1007/s10164-005-0174-2

Pratt-Phillips, S. E., Stuska, S., Beveridge, H. L., and Yoder, M. (2011). Nutritional quality of forages consumed by feral horses: The horses of Shackleford Banks. *J. Equine Vet. Sci.* 31, 640–644. doi: 10.1016/j.jevs.2011.05.012

Rubenstein, D. I. (1981). Behavioural ecology of island feral horses. *Equine Vet. J.* 13, 27–34. doi: 10.1111/j.2042-3306.1981.tb03443.x

Rubenstein, D. I. (1986). "Ecology and sociality in horses and zebras," in *Ecological Aspects of Social Evolution*, eds D. I. Rubenstein and R. W. Wrangham (Princeton, NJ: Princeton University Press), 282–302. doi: 10.2307/j.ctt7zvwgq.17

Rubenstein, D. I. (1994). "The ecology of female social behavior in horses, zebras, and asses," in *Animal Societies: Individuals, Interactions, and Organization*, eds P. Jarman and A. Rossiter (Kyoto: Kyoto University Press), 13–28.

Rubenstein, D. I. (2011). Family equidae (horses and relatives). *Handbook Mammals World* 2, 106–143.

Rubenstein, D. I., and Hohmann, M. E. (1989). Parasites and social behavior of island feral horses. *Oikos* 55, 312–320. doi: 10.2307/3565589

Rutberg, A. T. (1987). Horse fly harassment and the social behavior of feral ponies. *Ethology* 75, 145–154. doi: 10.1111/j.1439-0310.1987.tb00648.x

Zervanos, S. M., and Keiper, R. R. (1979). "Seasonal home ranges and activity patterns of feral Assateague Island ponies," in *Proceedings of a Conference on the Ecology and Behavior of Feral Equids*, ed. R. H. Denniston (Laramiepp: University of Wyoming), 3–14.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership