CORAL REEF RESTORATION IN A CHANGING WORLD: SCIENCE-BASED SOLUTIONS

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Editorial: Coral Reef Restoration in a Changing World: Science-Based Solutions

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Editorial on the Research Topic

Coral Reef Restoration in a Changing World: Science-Based Solutions

Coral reef ecosystems are impacted globally by anthropogenic and climate change, altering ecosystem functioning, and the goods and services reefs provide to societies (Bindoff et al., 2019). Further deterioration of the reef framework and decreases in reef-associated biodiversity are expected, necessitating rehabilitation responses. With the increase of anthropogenic impacts, scientists, conservationists, environmentalists, and decision-making authorities initially employed traditional conservation and rehabilitation approaches (such as additional marine protected areas, no use zones, etc.), and practices that aimed to reduce local stressors such as fisheries and tourism, following the rationale that these activities will lead to rehabilitation of reefs by natural recovery. Yet, this approach of 'passive restoration' has generally failed to achieve its goals (Rinkevich, 2008). As a result, more and more practitioners and scientists are opting for active reef restoration (Baums et al., 2019; Bindoff et al., 2019; Bayraktarov et al., 2020; Kleypas et al., 2021), where human activities directly foster the recovery of damaged reef ecosystems. Advances in fundamental science, further development of an applied tool-box (Vardi et al., 2021), and supplementary ecological engineering approaches (Rinkevich, 2021) are needed to help active restoration succeed.

In one way, the declaration of the Decade on Ecosystem Restoration by the United Nations has raised interest in the methods needed to implement best practices for maximum gain. In another way, the International Coral Reef Society (ICRS) has recently published a science to policy paper (Knowlton et al., 2021) describing a "plan to save coral reefs" where three main pillars are presented as equally important for corals to be retained: 1) mitigation of CO2 emissions; 2) mitigation of local pollution; and 3) active restoration. Our most important scientific society pointed out active restoration as one of the requirements for coral reefs to "survive". Yet a lack of restoration protocols, clear criteria for restoration outcomes and financial support posed significant obstacles to its maturation. Based on the above, this Research Topic entitled: 'Coral Reef Restoration in a Changing World: Science-based Solutions' aimed, to encourage and collect studies searching for innovative techniques and ecological engineering approaches in coral reef restoration programs, with an eye to current and anticipated stressors that affect coral reef ecosystems. The articles were spread across the following themes:

- Propagation and Husbandry
- Improved Outplanting Success
- Size Matters! Advances in Microfragmentation for Reef Restoration
- Building Resilient Reefs: Assisted Evolution and Genetic Influence on Performance and Harnessing Environmental Gradients
- Spawning a Future: Assisting Coral Recruitment Through Larval Husbandry
- Socio-economic Studies of Citizen Participation in Restoration Programs
- Species Ecology - Growth, Feeding, and Reproduction
- Monitoring Assessment Technology and Tools
- Models for Restoration and Management

With 116 researchers from 14 countries, the 19 articles in this Research Topic (https://www.frontiersin.org/research-topics/12642/coral-reef-restoration-in-a-changing-world-science-based-solutions#articles), reveal a broad spectrum of science-based coral reef restoration information from around the world—from Palau, Seychelles, Vietnam, Philippines, Kenia, Mozambique, China, Taiwan, Australia to Bermuda, United States, Mexico, Dominican Republic, France, to the United Kingdom (https://www.frontiersin.org/research-topics/12642/coral-reef-restoration-in-a-changing-world-science-based-solutions#authors). Authors belong to a wide range of organizations from universities, and research centers to Non-Governmental Organizations. The articles published here cover numerous subjects within the themes proposed in this Research Topic and have received worldwide attention— from Ukraine, Lithuania, Poland, Germany, Denmark, Russia, China, India, Australia, United States, France, Mexico, to Pacific islands such as French Polynesia, Mauritius, Reunion, Seychelles, Madagascar, or Maldives to mention a few. The top five countries from which articles have been viewed are the United States, Australia, China, the United Kingdom of Great Britain and Northern Ireland and Germany (https://www.frontiersin.org/research-topics/12642/coral-reef-restoration-in-a-changing-world-science-based-solutions#impact).

MONITORING ASSESSMENT TECHNOLOGY AND TOOLS

The Coral Reef Consortium Monitoring Working Group developed a guide to monitor coral reef restoration and to determine restoration success using two metrics (Goergen et al., 2020): Universal and Goal-Based Performance Metrics (GBPM). Four Universal Metrics were suggested (Landscape/Reef-level, Population-level, Colony-level, and Genetic/Genotypic Diversity) and five GBPM were addressed (Ecological Restoration, Socioeconomic, Event driven Restoration, Climate Change Adaptation, and Research). This Research Topic includes articles with both metrics. Hein et al., synthesized recent state of knowledge, providing information on current concepts changes in coral reef restoration, considering the goals, current methods, and the value of reef restoration in the face of climate change. The study further provided recommendations, including the implementation necessity of effective restoration planning/design; the need for defining of specific goals/objectives; more appropriated methods for specific goals/cost, effectiveness, and scalability in reef restoration methods, and four directions to restore coral reef ecosystems. Dao et al., stressed that in many cases, corals live close to their temperature limit, therefore, higher sea surface temperatures may follow with prolonged bleaching events, leading to coral death. Using climate change projections for the Cu Lao Cham-Hoi biosphere (they raised concerns that corals will face prolonged temperature stresses, calling for immediate actions. Dang et al., focused on the role of sea urchins roles in maintaining coral reef equilibria. Sea urchins are important because they can exert top-down control on algae, following the overfishing of other herbivores such as fishes and gastropods. They also revealed a relationship between coral juveniles' survivorship and sea urchin density. Cortés-Useche et al., stressed the importance of fish assemblages in restoration and presented an innovative method where first life stages of fishes are considered in reef regeneration. Protecting early life stages from predation in aquaria and delaying their release until they are juveniles may speed up habitat recovering processes. The authors demonstrated promising results to consolidate this method in the Caribbean Sea.

PROPAGATION AND HUSBANDRY

Coral reef restoration through larval rearing and sexually propagated juvenile corals is crucial for preserving coral reef ecosystem functions and services under global and local stressors (Baums, 2008; Hancock et al., 2021). Sellares-Blasco et al., studied a bottleneck in reef restoration, improved propagation, and husbandry, focusing on coral assisted fertilization, larval rearing/recruit propagation success in the Dominican Republic. Two years following inception, they developed an annual regional coral spawning prediction calendar, seeding >268,200 recruits in 1,880m^2 reef. Maneval et al., maricultured *Acropora cervicornis* fragments from different genets in two nursery types at shallow and deep-water depths over 6.5 months. While documenting high variation between genets, they recorded higher growth rates in the deep-water nurseries that had less biofouling, thus requiring reduced maintenance.

IMPROVED OUTPLANTING SUCCESS

Nowadays, most restoration efforts focus on outplanting corals from nurseries or freshly-pruned fragments to restore coral reefs, and outcomes depend on survival rates. Calle-Triviño et al., highlighted the importance of the improvement of degraded reefs' ecological functions. where coral restoration has been implemented Using *Acropora cervicornis* outplants into four-sites, they recorded increased fish biomass, coral cover, and structural complexity, providing much-needed evidence for active restoration's ecological benefits. Schill et al., have tested imaging spectroscopy from the Global Airborne Observatory (GAO) and reported transplants' survivorship rates at restored

sites (Dominican Republic, 3-7m depths) over 11 months. Results revealed that GAO-derived map products provided a quantitative and replicable method for selecting restoration sites characterized by increasing outplant survivals.

SIZE MATTERS! ADVANCES IN MICROFRAGMENTATION FOR REEF RESTORATION

The microfragmentation technique, allows small-sized ramets at the nubbin sizes to grow more rapidly compared to coral fragments of larger sizes. Papke et al., have employed an outdoor experimental setting with *Acropora palmata* microfragments, revealing differential effects of substrates (cement, ceramic) and genets on coral growth. Genet had a more substantial influence than substrate and coral growth on cement-substrates was better when compared to ceramic substrates, suggesting that both factors, genet and substrate should be considered. Koch et al., used a structured light 3D-scanner to evaluate surface-area (SA) measurements of living tissues over time, and developed a novel protocol for quantifying growth rate of fragmented living corals. Compared with the conventional 2D approach (photography and ImageJ analysis), they found that the 3D approach had some advantages but was slower and more expensive. Yet, it is more accurate for measuring SA for complex colony shapes.

BUILDING RESILIENT REEFS: ASSISTED EVOLUTION AND GENETIC INFLUENCE ON PERFORMANCE AND HARNESSING ENVIRONMENTAL GRADIENTS

Climate change affects reef coverage, function, and distribution of species. The question thus arises which species and genets to use when restoring reefs given that environmental conditions are expected to deteriorate for some time to come. Quigley et al., bred corals with variable heat-tolerance, by crossing surviving colonies from the 2016-2017 mass bleaching events among three regions of the Great Barrier Reef (GBR) and followed survival/growth of offspring over 217 days. Crosses within regions had the highest survival rates, suggesting local adaptation. Yet, some between-region crosses grew faster, demonstrating that breeding corals across latitudes may provide a viable approach to increase heat tolerance of restoration stock. Caruso et al., focused on the importance of stress-tolerant species of corals to ensure long-term ecosystem functioning and viability, highlighting the potential costs as well as the existing gap of knowledge associated with their approach. Humanes et al., considered the question if it makes sense to restore reefs with the same coral stock that is currently failing to thrive. Knowing that increasing water temperatures will continue for decades to come, restoration practitioners may rely on selectively breed corals for higher temperature tolerance. Humanes et al., further laid out a framework for how selective breeding may be carried-out, providing data on survivorship and growth of selectively bred corals.

SPAWNING A FUTURE: ASSISTING CORAL RECRUITMENT THROUGH LARVAL HUSBANDRY

While unmitigated CO_2 emissions and the warming oceans they cause remain the biggest threat to reefs world-wide, the lack of coral recruits has emerged as a prime obstacle to coral recovery and adaptation. Harrison et al., reported that "increased coral larval supply enhances recruitment for coral and fish habitat restoration" and that supplying larvae directly to a degraded reef may reestablish breeding populations, increasing coral cover and enhancing fish abundance. Randall et al., experimented with refugia for *Acropora tenuis* recruits on artificial settlement devices, with the aim of reducing grazing and predation pressure. Results revealed increased recruit survival on tiles with wide slits versus lattice grid or flat control tiles. Such a design prevents predation, grazing, and sediment accumulation, which are some of the significant causes for post-settlement coral mortality. Luo et al., examined the genetic diversity and gene flow patterns in *Porites lutea* populations from 9 to 22 degrees latitude in the South China Sea, a less studied region despite its abundant reefs. As elsewhere in the Pacific, gene flows was high among *P. lutea* populations. Instead, the authors found that that genetic diversity correlated with temperature gradients.

SPECIES ECOLOGY - GROWTH, FEEDING, AND REPRODUCTION

Understanding the ecology of coral species should also be considered in conjunction with local/global stressors. VanWynen et al., studied whether inter-species hybrids have accelerated skeletal growth during coral restoration. They found variation in growth rates among two Caribbean Acropora species and their F1 hybrid at three coral nurseries in the Bahamas. The F1 hybrids grew faster than the two parental species, suggesting that the F1 hybrid represents an alternative to repopulate reefs requiring enhanced coral growth. Authors also show that growth rates vary across locations, suggesting that some sites harbor better conditions to enhance coral growth and develop nurseries.

MODELS FOR RESTORATION AND MANAGEMENT

There has been a rapid development of new tools in modeling restoration and management strategies that opens new avenues to improve restoration outcomes. Modelling is necessary to consider the complex influences of global climate impacts and multiple local stressors and help integrate the recent accumulation of massive data

sets. Feng et al., assessed the potential environmental impacts of artificial upwelling (AU) over large areas in the GBR, South China, and Hawaiian regions *via* a 3D Earth System model. They obtained variable results from upwelling layer models (from 130 to 550 m) and showed that AU can effectively reduce sea surface temperature (SST) and degree heating weeks (DHW) and slow future coral bleaching events. However, when water is upwelled from a deeper layer (550m) and at high rates, it may cause severe risk to corals, revealing the importance of regional models together with experimental studies on the effects of UA on coral reef systems. Coral reef restoration research further generates an enormous amount of essential information to be appropriately and systematically organized and stored. Moura et al., presented the Coral Sample Registry (CSR), an online resource that establishes and integrates diverse coral restoration data sets. The CRS concept is based on fostering dialogues among restoration practitioners, federal and state agency managers, and researchers for centralizing information on sample collection events.

In summary, this Research Topic showcases the fast development of tools to support active coral reef restoration and highlighted the importance of a comprehensive tool-box. Some of the approaches are more general and can be applied to all most reefs worldwide, and others are more restricted to specific coral taxa and/or reef areas. Coral reef restoration emerged over the last three decades and is now a fully recognized field that is aiming to preserve functional reef diversity until CO_2 emissions have been reduced and the rate of ocean warming slows.

AUTHOR CONTRIBUTIONS

J-AG and BR wrote the first draft of the manuscript. BR, IB, J-AG, CP, ATB, SR edited final manuscript. All authors contributed to summaries and manuscript revision, read, and approved the submitted version.

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ORIGINAL RESEARCH
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Modeling Coral Bleaching Mitigation Potential of Water Vertical Translocation – An Analogue to Geoengineered Artificial Upwelling

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Artificial upwelling (AU) is a novel geoengineering technology that brings seawater from the deep ocean to the surface. Within the context of global warming, AU techniques are proposed to reduce sea surface temperature at times of thermal stress around coral reefs. A computationally fast but coarse 3D Earth System model (3.6° longitude × 1.8° latitude) was used to investigate the environmental impacts of hypothetically implemented AU strategies in the Great Barrier Reef, South China Sea, and Hawaiian regions. While omitting the discussion on sub-grid hydrology, we simulated in our model a water translocation from either 130 or 550 m depth to sea surface at rates of 1 or 50 m^3 s^{-1} as analogs to AU implementation. Under the Representative Concentration Pathway 8.5 emissions scenario from year 2020 on, the model predicted a prevention of coral bleaching until the year 2099 when AU was implemented, except under the least intense AU scenario (water from 130 m depth at 1 m^3 s^{-1}). Yet, intense AU implementation (water from 550 m depth at 50 m^3 s^{-1}) will likely have adverse effects on coral reefs by overcooling the surface water, altering salinity, decreasing calcium carbonate saturation, and considerably increasing nutrient levels. Our result suggests that if we utilize AU for mitigating coral bleaching during heat stress, AU implementation needs to be carefully designed with respect to AU's location, depth, intensity and duration so that undesirable environmental effects are minimized. Following a proper installation and management procedure, however, AU has the potential to decelerate destructive bleaching events and buy corals more time to adjust to climate change.

Keywords: coral bleaching, ocean biogeochemical modeling, artificial upwelling, global warming, sea surface temperature rise, heat waves, earth system modeling

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INTRODUCTION

Shallow-water coral reefs sustain some of the most biodiverse ecosystems on the planet, providing numerous ecosystem services and values to both humans and the environment (Deloitte Access Economics, 2013). Thriving in the euphotic zone at tropical latitudes, corals face increasing temperature-induced bleaching events (Aronson et al., 2002; Hughes et al., 2018). During

bleaching events the corals expel the zooxanthellae (*symbiotic algae*) living within their tissue as a response to the high temperature stress, leaving the white coral skeletons behind. Because zooxanthellae provide, through photosynthesis, most of the energy required by their hosts, the loss of zooxanthellae can lead to coral starvation and death, and ultimately to reef degradation. According to the current tendency of economy and population growth, Earth's temperature is predicted to still increase across 1.5°C warming target by the end of 21st century, even if current climate mitigation efforts are adopted (Raftery et al., 2017). Sea surface temperature (SST) will rise in the next decades and increase the probability of extreme marine heat events (Frölicher and Laufkötter, 2018) and hence, the frequency and intensity of mass coral bleaching events contributing to reef degradation worldwide (Hughes et al., 2017). Consequently, climate change adaptation measures that could provide a longer time window for corals to cope with rising temperature, are needed along with the climate mitigation efforts.

Natural upwelling, which introduces cooler sub-thermocline water into shallow reef areas, can effectively cool surface waters, and thus provide temporal refugia for coral reefs when upwelling coincides with thermal events (Jiménez et al., 2001; Bayraktarov et al., 2013; Chollett and Mumby, 2013; Wall et al., 2015). Upwelling can also be achieved with a geoengineering technology called artificial upwelling (AU). Powered by either ocean waves, solar or ocean thermal energy, AU is designed to pump seawater adiabatically from depths below the thermocline to the sea surface, and discharges the upwelled water via a floating platform (Kirke, 2003). So far, AU has been considered primarily to increase surface ocean primary productivity by pumping up nutrient-rich deep water, to either enhance CO_2 sequestration from the atmosphere and thereby mitigate global warming (Liu and Jin, 1995; Kirke, 2003) or to increase the yield of fisheries (Viúdez et al., 2016). AU, however, will also cool the ocean surface by up to several degrees Celsius (Oschlies et al., 2010) inferring that AU may be a viable tool to reduce heat stress for corals.

In order to gain a first understanding on the effect of AU on reef waters and the surrounding system, we simulated the implementation of vertical tracer translocation as an analog to AU (hereafter for descriptive convenience, we do not distinguish between AU and water translocation unless specifically noted), in three major coral reef systems in the Indo-Pacific (Great Barrier Reef, South China Sea, Hawaiian islands) from year 2020 to 2100, using the University of Victoria Earth System Model (UVic). The model uses a spatial and temporal resolution that allows a cost-effective representation of the complex evolution of three-dimensional (3D) ocean hydrology and biogeochemistry under future climate change. Although this rather coarse model does not provide definitive answers regarding AU implementation on small local scales, its main advantage lays in the simultaneous exploration of multiple parameters that may be altered by AU in the context of global warming, which is the main goal of this study. Therefore, we investigated the potential efficiency of AU in mitigating coral bleaching by analyzing its effects on SST and accumulated heat stress in the three test regions. We also assessed the potential side effects of AU practices in terms of changes in ocean circulation and marine biogeochemistry (i.e., ocean temperature, salinity, nutrients and calcium carbonate saturation states et al.). We hypothesized that AU will reduce SST and coral bleaching effectively; however, depending on the implementation strategy of AU, some undesirable side effects might occur. Possible side effects include severe reduction in temperature (overcooling), salinity changes, acidification and nutrient-enrichment, which may affect the biogeochemistry and hydrology of reef waters as well as of neighboring water masses. Depending on the severity of these changes and the susceptibility of ecosystems, this may entail substantial pressure on coral health and ecosystem functioning (Kleypas et al., 1999; Guan et al., 2015). For example, excessively cooled water can have similarly negative effects on the corals' metabolism as thermal stress (Saxby et al., 2003; Lirman and Manzello, 2009; Kemp et al., 2011; Roth et al., 2012). Seemingly, the enrichment of dissolved inorganic carbon (DIC), and subsequent reduction of ocean pH, can impair coral growth (e.g., calcification) (Schneider and Erez, 2006). Increased nitrogen and phosphorus input may promote macroalgae growth, which could outcompete corals (McCook, 1999; Szmant, 2002) and may also affect coral health (Wiedenmann et al., 2012). Generally speaking, any drastic change in the physical and chemical properties of water masses has the potential to affect exposed organisms, which can ultimately alter ecosystem composition and functioning. Therefore, before AU can be implemented, we need to gain a holistic understanding of the benefits and risks of AU. The first step towards that goal is to numerical model effect of AU on the water properties, which will provide some initial insight into answers for the following questions:

(*i*) Can AU reduce the duration and intensity of heat stress in coral reef systems?
(*ii*) What are the possible adverse environmental effects of AU?
(*iii*) What are the optimal AU operational strategies that would lead to successful bleaching mitigation, with minimum harmful impacts on environment and acceptable costs?

MATERIALS AND METHODS

Model Description

Model simulations were performed with the coarse-resolution Earth system model University of Victoria Earth System Climate Model (UVic) version 2.9 (Weaver et al., 2001). The ocean component of UVic is formed by a general ocean circulation model (Pacanowski, 1996) coupled to a nutrient–phytoplankton–zooplankton–detritus (NPZD) module and a marine carbon cycle, so that 3D marine biogeochemical processes can be represented. The terrestrial module of UVic is modified from the Top-down Representation of Interactive Foliage and Flora Including Dynamics (TRIFFID) vegetation model (Meissner et al., 2003). Within UVic, the atmosphere is described by an energy–moisture balance model (Fanning and Weaver, 1996), and processes related to sea ice are characterized with a sea

ice module (Bitz and Liscomb, 1999). The oceanic component has a horizontal resolution of 3.6° longitude × 1.8° latitude. A full seawater column has 19 layers, with a gradually coarser resolution starting from 50 m thickness near the surface, up to 500 m thickness at the deepest layer near the ocean floor. The UVic's isopycnal mixing follows Gent-McWilliams parameterization (800 m^2 s^{-1} for diffusivities) for mesoscale eddies and Bryan-Lewis scheme for the vertical tracer diffusivity (0.3 – 1.3 cm^2 s^{-1})(Weaver et al., 2001). Ocean wave states are not considered for air-sea heat, momentum, gas, aerosol and moisture fluxes, hence no wave excitation energy accounted. The simulated SST and oceanic pCO_2 were evaluated against data from the World Ocean Atlas (Locarnini et al., 2013), and the Surface Ocean CO_2 Atlas (Landschützer et al., 2014; Feng et al., 2016). This evaluation showed that UVic simulates sea surface pCO_2 within the range of observations, and the resulting SST was also well within range of date-error bands from the Coupled Model Intercomparison Project (CMIP) models (Wang et al., 2014; Feng et al., 2016). UVic has been previously used to investigate the climate mitigation potential of regionally implemented ocean alkalinization under a business-as-usual emission scenario, in the Great Barrier Reef, Caribbean Sea, and South China Sea (Feng et al., 2016). The robustness of its representation of the equatorial current system in the upper Pacific Ocean has also been demonstrated (**Supplementary Text S1** and **Figure S1**). Despite the relatively coarse spatial resolution of UVic, the results of previous modeling studies (Oschlies et al., 2010; Keller et al., 2014) indicate it is an effective tool to simulate AU for providing a first understanding on how AU may effect SST, biogeochemistry and hydrology of ocean waters and presenting referential information to assist future assessment on this topic.

In this study, the fast and inexpensive global UVic model was chosen over slow and expensive regional models, since it is an efficient tool to gain some initial insights into how water translocation can affect the physical characteristics and the biogeochemistry of surface waters and the water column. To the best of our knowledge, even for very fine-resolution biogeochemical models, simulating detailed ocean dynamics and biogeochemistry near shallow coral-inhabiting waters remains a challenge because of the complex nature of shallow-water environments (Zhang et al., 2012; Lessin et al., 2018) and the difficulties in coupling ocean currents, waves, and biogeochemistry within numerical models (Mongin and Baird, 2014). Coupling AU hydrological modules with a regional biogeochemical model imposes another technical challenge. Such sophisticated numerical coupler has only been seen from environmental assessments provided by dedicated enterpriser teams (Pat Grandelli et al., 2012). The UVic model instead, allows simulations of complex 3D physical and biogeochemical properties on large spatial scales, from which key information is transformable and valuable to understanding smaller scale (localized) impacts where knowledge on 3D dynamics are limited. Therefore, with carefully designed simulations to minimize possible caveats, UVic can be employed as an useful tool to studying ocean interventions at both global and regional scales (Bonan and Doney, 2018), especially to gain a first understanding in a timely and efficient fashion (Keller et al., 2014; Feng et al., 2016).

Regions of Interest

We chose experimental areas larger than 100,000 km^2 for testing AU to fully utilize the current advantage of UVic that is capable of simulating marine biogeochemistry across large ocean basins efficiently and robustly in three dimensions. With this large-scaled implementation of AU and the global coverage of UVic, mesoscale ocean dynamics, such as eddies, are presumed insignificant in modulating AU plumes, hence not necessarily less accurate than high-resolution models (Petoukhov et al., 2005). Accordingly, the trajectories of upwelled water over long distances are obtainable for investigating the impacts of AU beyond our regions of interest. Those large-scaled simulations could also be viewed as ambitious AU implementation scenarios with extremely large amount of water being manually relocated, which is scientifically meaningful to investigate the possible environmental constraints. Those important features usually cannot be offered by regional models in a cost-effective manner.

The Great Barrier Reef (GBR; 140.4°E–154.8°E and 9.0°S–27.0°S, 1.7 × 10^6 km^2), South China Sea (SCS; 104.4°E–129.6°E and 0°N–23.4°N, 5.2 × 10^6 km^2), and Hawaii (HWI; 176.4°E–151.2°W and 16.2°N–27°N, 4.0 × 10^6 km^2) were chosen as the test regions (**Figure 1** and **Supplementary Figure S2**). Corals from these regions have experienced bleaching in the past (Marshall and Baird, 2000; Jokiel and Brown, 2004; Li et al., 2011; Waheed et al., 2015; Hughes et al., 2017). GBR, SCS and HWI together contain more than 50% of the coral reef locations in the world, while GBR and SCS have both been used as test regions in previous modeling experiments to investigate the implementation of other geoengineering strategies (Feng et al., 2016). GBR and SCS represented continental marginal seas, whereas HWI was selected as an open ocean setting. Our aim was to investigate the possible environmental changes caused by AU implemented in regions with distinct hydrographic and topographic patterns.

Simulating AU and Model Run Setup

Since the UVic model can simulate large scale ocean warming under climate change, we analyzed the environmental changes under the long-termed future warming trajectory for cases without and with the implementation of AU. We focused on the variance between different AU scenarios and the control run (no AU) instead of analyzing the absolute values calculated from the AU simulations. By investigating the differences in the model outputs of control versus AU runs, we were able to examine the environmental changes caused by AU while minimizing the model-oriented biases. The model was spun-up for 10,000 years from the preindustrial period with a prescribed atmospheric CO_2 concentration and then ran for another 200 years to year 1765 without it to avoid climatic drifts. It was run further from year 1765 to 2005 under historical CO_2 emissions forcing, followed by another period of simulation until the end of 2099 under the Representative Concentration Pathway 8.5 (RCP 8.5) high-CO_2-emission scenario (Meinshausen et al., 2011) as

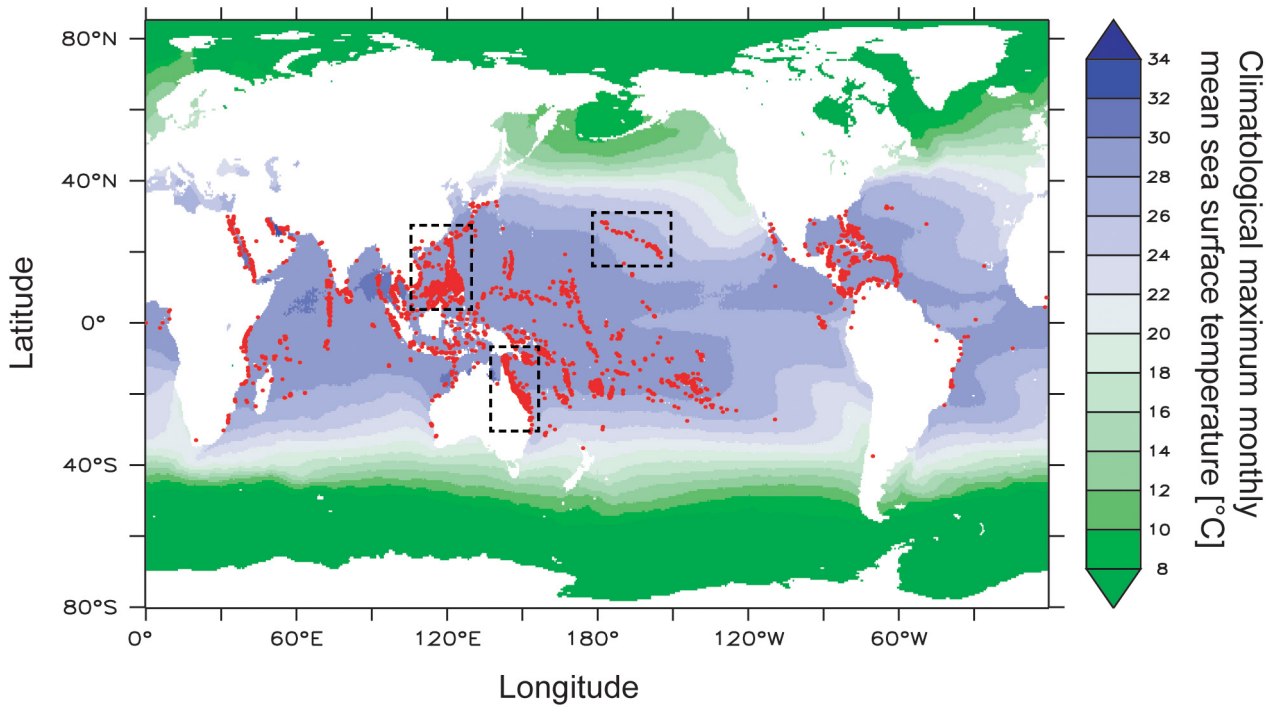

FIGURE 1 | Map containing grid cells in which artificial upwelling (AU) has been implemented (marked with black boxes), overlaid onto the maximum monthly mean (Max-MM) sea surface temperature (SST) based on 1985–1993 climatology data. Max-MM SST data was downloaded from *Coral Reef Watch* (https://coralreefwatch.noaa.gov/satellite/hdf/index.php), and the coral reef locations, marked as red spots, were obtained from *ReefBase* (http://www.reefbase.org/main.aspx).

extensive forcing. We chose the model simulation from 2020 to the end of 2099 for the control run (no AU) and for the AU simulations.

As UVic cannot resolve the sub-grid hydrodynamics rigorously, the simulation of AU cannot comply to parameterizations in respect to water lifting, discharging and remixing from *in situ* experiments (White et al., 2010). Therefore, in this study we described AU process as a special oceanic tracer (temperature, salinity, et al.) translocation from one depth to the surface with no detailed characterization of the upwelling hydrology, and used flow units (Sv) to constrain the assigned AU rates. This approach could impose uncertainties given the simplification of crucial hydrological processes, thus future studies are required to test the validity of such simplification as well as suggesting improvements to overcome such common caveat. With currently no related studies available to validate or invalidate such a method, we hereby use this method to investigate AU's environmental impacts, focusing on crucial parameters like temperature, nutrients and aragonite saturation state while omitting the hydrodynamic component. Currently proposed AU prototypes pump seawater at rates ranging from 1 to 50 m^3 s^{-1} (Liu and Jin, 1995; Kirke, 2003; Trench et al., 2004) with pipes that can extend down to several thousand meters below the sea surface (Matsuda et al., 1999; Kirke, 2003). Since the UVic model does not resolve the sub-grid hydrology below its resolution, simulating AU by 1 m^3 s^{-1} at a geographic density of 1 device per km^2 is equivalent to simulating it by 0.01 m^3 s^{-1} at a density of 100 devices per km^2. We chose the upper and lower ends of potential AU upwelling rates (1 and 50 m^3 s^{-1}), and used the suffix "*low*" (1 m^3 s^{-1}) or "*high*" (50 m^3 s^{-1}) in the names of the model runs to indicate the upwelling rate applied. It is noted that in practice, such upwelling rates might not lead to full surface mixing as the AU plumes can be diluted and sink rapidly (Pan et al., 2019). In the model, we set that AU operating at rate 1 or 50 m^3 s^{-1} were virtually deployed uniformly at density of one device per square kilometer, and such scenario also refers to more realistic implementation strategies under which upwelling rates and density at localized scales could be adjusted as long as the area-integrated flow rate match the values given in Sv (1.7, 5.2 and 4.0 Sv for "*low*" runs, and 85, 260, 200 Sv for "*high*" runs). We set the local seawater to be upwelled vertically from a depth of 130 or 550 m below the sea surface, therefore the suffix "*shallow*" for 130 m or "*deep*" for 550 m was added to the names of these AU runs. If the seawater column was shallower than these two depths, the water was upwelled from the deepest ocean layer possible. We also assumed that the upwelled water was adiabatically transported, with no changes in its original physical or chemical properties during the translocation process (Oschlies et al., 2010). The four AU scenarios, namely "*shallow_low,*" "*shallow_high,*" "*deep_low,*" and "*deep_high,*" as analogs to AU implementations, and a control run without AU, were simulated to examine the effect of AU on temperature, hydrodynamics and biogeochemical properties of ocean water. This is expected to provide some

insight into how different AU scenarios may affect coral reefs during thermal stress.

Characterization of the Occurrence and Severity of Coral Bleaching

Coral bleaching is predominantly induced by accumulated heat stress over a given period of time. Corals are reported to be stressed under abnormally elevated sea temperatures (defined as the *bleaching threshold*), which is commonly found to be 1°C higher than the maximum mean temperature during the climatological summer months (Max-MM) (Ncrw, 2000). To quantify heat stress, the metric "degree heating weeks" (DHW; unit:°C-week) is used, which is calculated by adding the positive weekly mean temperature anomaly referenced to *bleaching threshold* over a period of 84 days (12 weeks). Coral bleaching events are most likely to appear when DHW starts to exceed 4°C-weeks, while areas with DHW higher than 8°C-weeks have a high probability of bleaching and consequent coral mortality (Liu et al., 2006). Hence, *bleaching threat level 1* (BL-LV1) is defined by DHW of 4°C-weeks to 8°C-weeks, and *bleaching threat level 2* (BL-LV2) by DHW > 8°C-weeks. In this study, we used a modified version of DHW, namely "degree heating 5 days" (DH-5d; unit:°C-5 days), to characterize the occurrence, duration, and intensity of coral bleaching threat. DH-5d instead of DHW was used, because the UVic model worked with a 5-day mean temperature anomaly instead of a weekly mean. Accordingly, the corresponding bleaching alert levels were 5.7°C-5 days and 11.33°C-5 days for BL-LV1 and BL-LV2, respectively.

Max-MM SST of each study regions was derived from satellite-based datasets from year 1985 to 1993 (excluding 1991 and 1992. Liu et al., 2006) downloaded from CoralWatch (Ncrw, 2000), and the following *bleaching thresholds* were calculated for each region*:* 29.57°C for GBR, 30.50°C for SCS, and 28.30°C for HWI. The model results were presented as time series of area-averaged 5-day SST (5 day-SST) values for each region from 2020 to 2099. In the next step, SST positive anomalies were calculated by subtracting the *bleaching threshold* of each region from the respective 5 day-SST values. Time points with no positive anomaly were assigned to the value zero. Finally, the sum of the calculated SST anomalies over a period of 85 days were calculated (i.e., 85 days adjusting for the temporal interval of UVic, approximately 12 weeks) from the beginning of 2020 to the end of 2099, providing a time series of DH-5d for each region. These procedures were applied to each of the five model runs (one control and four AU runs).

Coral bleaching and subsequent coral death do not only occur during high temperature anomalies, but can also during abnormally cold periods (Hoegh-Guldberg and Fine, 2004; Lirman et al., 2011). Cold water stress event are, however, much less studied than heat stress events, therefore no comparable bleaching thresholds exist for cold stress events. To estimate potential stressful conditions due to overcooling, we simply compared the area-averaged SST time series for the three test regions (GBR, SCS, and HWI) with the coldest monthly mean SST among all 12 months for the UVic climatological period 2000–2020 (referred as Min-MM SST; area-mean values: 24.74°C for GBR, 26.2°C for SCS, and 23.01°C for HWI). We assumed that corals experiencing temperatures below Min-MM SST could be stressed and calculated the number of days that would undergo < Min-MM SST conditions for all model runs. This provides an estimate of potential AU-induced cold water stress.

Characterization of the Environmental Changes Driven by AU

Besides SST, we also examined the AU-induced changes in sea water salinity, aragonite saturation state (Ω-Arag), and nutrient concentration (e.g., nitrate), at the implementation sites and in surrounding areas. These properties are known as important environmental drivers for the functioning of various coral reef organisms as well as for ocean dynamics (e.g., currents). As for temperature, thresholds of these parameters are strongly dependent on the history of local conditions to which organisms and communities are adjusted. However, since these thresholds are much less well defined than temperature thresholds, we used conditions that have been describe as the boundaries of shallow water coral reef habitat distribution worldwide (Kleypas, 1997). This includes a lower and upper salinity threshold of 28.7 and 40.4 PSU, a lower Ω-Arag threshold of 2.82, and an upper nitrate concentration threshold of 4.51 μmol L^{-1} (Guan et al., 2015). Those thresholds roughly frame the conditions in which tropical shallow water corals exist, and we therefore used them as a reference to discuss AU-induced environmental perturbations and their potential effect on corals and coral reefs.

RESULTS AND DISCUSSION

The model results provide insights on the effects of simulated AU on a very large spatial scale (10^6 km^2-scale). While, in practice AU would be implemented on much smaller scales (likely < km^2-scale), the model results can still provide a much required baseline of understanding about how AU may change temperature regimes as well as other parameters that may or may not cause unwanted side effects. Therefore, in the following, we will discuss the knowledge gained from the large-scale model in the context of small-scale/localized AU in coral reefs, assuming that similar dynamics of flow patterns and water mixing occur on large and small spatial scales, while keeping in mind the existing uncertainties from other high-resolution models in simulating coral reef ecosystems.

Impact of AU on SST and Coral Bleaching Threat

Overall, the model results showed that AU reduced SST markedly and hence the number of days of thermal stress. Furthermore, on the 80-year scale, AU caused significant reduction in the occurrence of bleaching events, assuming that bleaching thresholds are not changing over time. More specifically, under control conditions, the model predicted an increase in the area-averaged annual mean SST (aa-SST) over the next 80 years of 1.9–2.4°C for the three test regions. Under simulated year-round

upwelling, the model predicted the following reductions in aa-SST compared with that in the control run by the end of this century (year 2099): *shallow_low* by 0.06 – 0.2°C, *shallow_high* by 0.2 – 1.0°C, *deep_low* by 1.5 – 2.3°C, and *deep_high* by 4.6 – 8.9°C (**Supplementary Figure S3**). AU induced reductions in SST resulted in a reduction of number of days under BL-LV1 and BL-LV2 conditions (**Figures 2A–C**). Under control conditions and across the entire 80-year period from 2020 to 2099, predicted climate change resulted in approximately 12,145 (GBR), 10,440 (SCS), and 430 (HWI) days (**Supplementary Figure S4**) under *bleaching threatening* (BL-LV1&2, including BL-LV1 and BL-LV2) conditions, implying that bleaching was likely to occur. AU from "*shallow_low*" reduced the duration of BL-LV1&2 conditions to 11,750 (GBR), 7,115 (SCS), and 1,25 (HWI) days. The period of BL-LV1&2 conditions for the "*shallow_high*" model run were 290 (GBR), 775 (SCS), and 0 (HWI) days. The values of DH-5d with "*deep_low*" remained zero in SCS and HWI once AU was firstly started after year 2020 hence no BL-LV1&2 days at all for those two regions, whereas duration of BL-LV1&2 for GBR was 1,325 days. Intense AU implementation within "*deep_high*" produced very strong cooling effects and values of DH-5d remained zero for all regions after 2021, therefore no coral bleaching events due to heat stress were expected. The model showed, that depending on the AU settings, bleaching threat is reduced as well as delayed. While the "*shallow_low*" scenario delayed the occurrence of BL-LV1 condition by < 1 (GBR), 12 (SCI), and 4 years (HWI) after the year 2020, the "*shallow_high*" scenario delayed it by 74 years (GBR), 52 years (SCS), and 4 years (HWI) (**Supplementary Figure S4**). Both, "*deep*" scenarios completely eliminated BL-LV1 condition over the modeled 80-year period, with the exception of "*deep_low*" at GBR (delay of 62 years) (**Supplementary Figure S4**). These results imply that increasing upwelling rate and depth increase the effectiveness of AU in mitigating coral bleaching caused by heat stress.

Although AU reduced SST in all tested areas, the distinct features of these three regions, especially in their local topographies and thermoclines, led to noticeable differences in the AU-induced temperature perturbations and the changes in vertical stratification over time (**Supplementary Text S2**). In the three regions from 2020 to 2021, aa-SST dropped by (*i*) 0.13 (GBR), 0.17 (SCS), and 0.11°C (HWI) for "*shallow_low*" (*ii*) 1.07, 1.15, and 0.74°C, respectively, for "*shallow_high*", (*iii*) 1.04, 1.59, and 1.13°C, respectively, for "*deep_low*", (*iv*) and 9.52, 10.09, and 7.97°C, respectively, for "*deep_high*" (**Figure 2D**). SCS experienced the strongest reduction in temperature under all four AU scenarios. This is because within the UVic model, SCS was treated as a semi-enclosed region, where the water exchange with other ocean basins was lower compared to GBR and HWI (**Supplementary Figure S2**; Feng et al., 2016). The comparatively strong temperature gradient between the sea surface and the deep water (Δ temperature between surface and 550 m depth: 20.02°C in SCS, 17.29°C in GBR, and 15.49°C in HWI) also contributed to the strong cooling effect of AU at SCS. Furthermore, we observed that due to AU, the original warm sea surface water downwelling at and near the sites of AU implementation. Subsequently, the water temperature near the AU water source warmed leading to a gradually weakening of the vertical temperature stratification and hence reduced AU efficiency (**Figures 2E–G**).

The AU-induced reductions in SST within the three test regions were remarkable, and periods with 5 day-SST lower than Min-MM were seen in the model runs "*deep_low*" and "*deep_high*" (**Figure 3**). A particularly strong reduction in SST occurred in the "*deep_high*" scenario, where within the first 2 weeks after AU initiation 5 day-SST decreased to levels as low as 18°C or slightly lower (**Figures 3A–C**), while similar decline was much less severe (< 0.5°C) in the "*shallow_low*" and "*shallow_high*" scenarios (**Figures 3A–C**). The extent to which such overcooling will harm corals cannot be provided by our model. However, it has been shown that corals are susceptible to cold-water stress (Saxby et al., 2003; Hoegh-Guldberg and Fine, 2004; Howells et al., 2013), and tropical shallow water corals do not typically exist in waters below 18°C (Kleypas et al., 1999). For example in the GBR, corals which were transplanted to a region whose temperature minimum was 1.1°C cooler than the original region experienced a mortality rate of 40% (Howells et al., 2013). Therefore, we conclude that pronounced water cooling, with reduction of up to 7.97°C (**Figures 3A–C**) in "*deep_high*", is very likely stressful for corals that are not used to such temperature drops. Therefore, such extreme AU scenarios need to be avoided. This result demonstrates that choosing an appropriate upwelling rate and depth of water source with respect to temperature, are critical for AU implementations in order to minimize the impact of overcooling.

There are some key limitations to the model, which need to be considered when interpreting the results of the model runs. The UVic model, like many CMIP models, is biased in representing ocean temperature. In the GBR and SCS regions, the model generated an SST offset of up to + 0.8°C in reference to *in situ* observations, while the offset was up to -0.5°C in HWI (**Supplementary Figure S4** from Feng et al., 2016). Such representation was similar to most CMIP models and hence it was used in this study (Wang et al., 2014). However, because we used observed temperature datasets to calculate Max-MM SST as thermal thresholds, our test regions were warm (GBR and SCS) and cool-biased (HWI). Consequently, the DH-5ds heat stress levels generated by the model were slightly overestimated (GBR and SCS) or underestimated (HWI). Furthermore, in reality, massive bleaching events are often connected to large scale climate phenomena such as ENSO (El Niño-Southern Oscillation) events (Aronson et al., 2002; McGowan and Theobald, 2017). The atmospheric component of the UVic model, however, is modulated by an energy–moisture equation, and therefore it is not able to simulate dynamic wind feedbacks as well as large-scaled air-sea coupling. Consequently, we cannot capture climate phenomena such as ENSO, which can trigger marine heat waves in some regions. While the development of ENSO under future climate changes is still uncertain (Guilyardi, 2006; Wang et al., 2019), the majority of research tends to suggest an increase of its intensity and frequency in the future (Cai et al., 2015; Wang, 2018). Moreover, simulating the detailed impacts of ENSO is also challenging for the climate modeling community, especially in

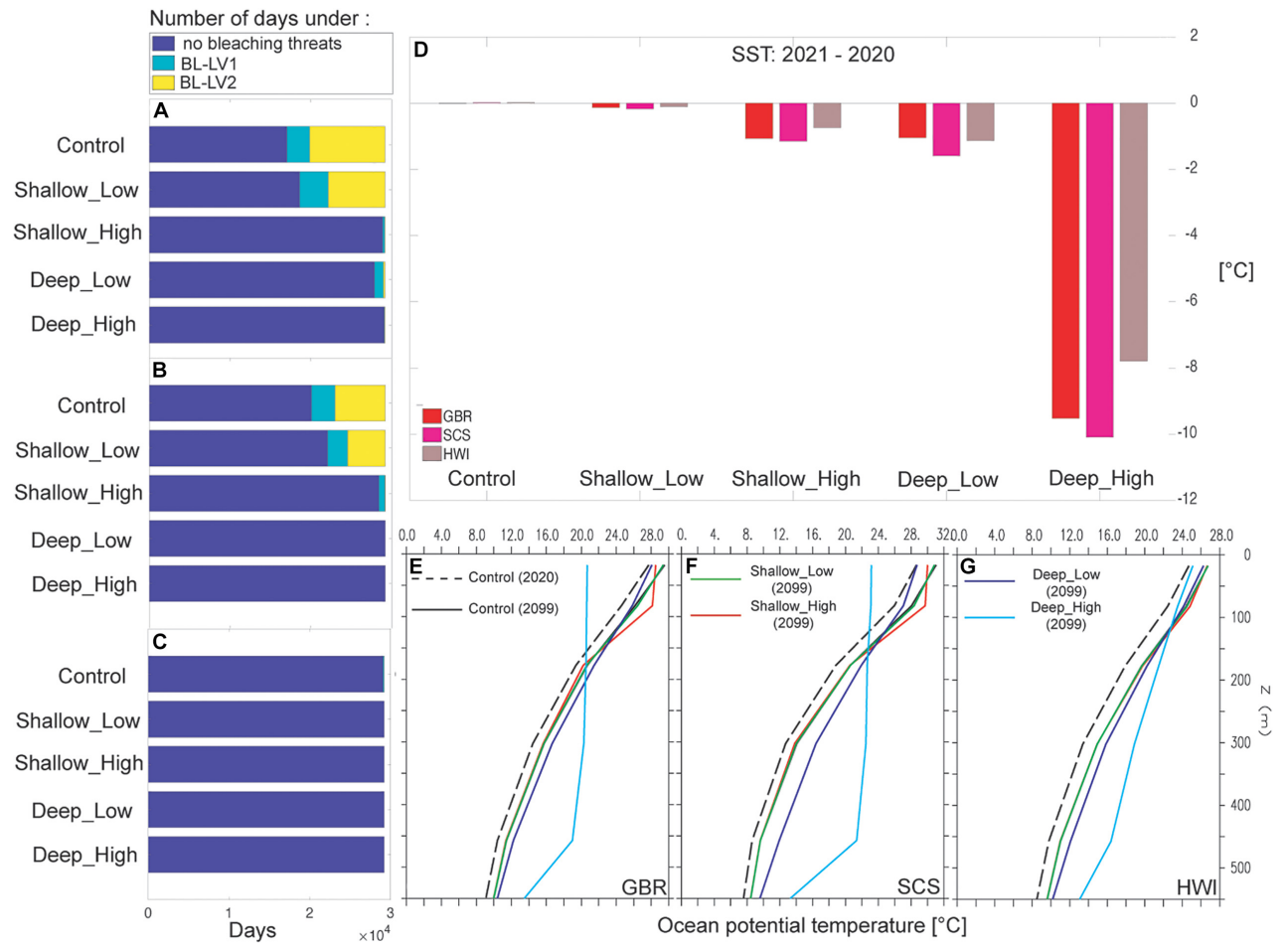

FIGURE 2 | Results of model runs for the Great Barrier Reef (GBR), South China Sea (SCS), and Hawaii (HWI) regions: The panels show, for the end of 2099, the accumulated number of days under different coral bleaching threat levels for region GBR **(A)**, SCS **(B)**, and HWI **(C)**; area-averaged difference in annual mean sea surface temperature (SST) between 2021 and 2020 **(D)**; and area-averaged annual mean sea potential temperature for depths to 550 m **(E–G)**.

our selected regions (Collins et al., 2010; Cai et al., 2015). In this study, we prepared model simulations for more than 50 years to generate SST deviations that resemble marine heat waves thus capable of triggering mass bleaching. By this we can provide meaningful results that help to identify the overall potential utility of AU in mitigating coral bleaching as well as knowledge gaps and side effects that need to be addressed by future studies.

Impact of AU on Salinity, Ω-Arag, and Nitrate Concentration

The model control run predicted a change of less than 0.1 PSU in area-averaged annual mean sea surface salinity (aa-SSS) by the year 2099 (**Figure 4**), resulting in 34.65 (GBR), 33.55 (SCS) and 35.17 (HWI) PSU. The effect of AU on aa-SSS varied greatly among the three test regions and four model runs in response to the different halocline structures and AU settings. For all three regions in year 2020, aa-SSS at the sea surface, 130 and 550 m depth, were *(i)* 34.80, 35.13, and 35.04 PSU respectively for GBR, *(ii)* 33.75, 34.16, and 34.61 PSU for SCS, and *(iii)* 35.16, 35.14 and 34.43 PSU for HWI (**Supplementary Figure S5**). Consequently, AU implementation increased aa-SSS in GBR and SCS by up to 0.78 PSU, and decreased aa-SSS in HWI by up to 0.52 PSU over the 80-year period (**Figure 4**). Furthermore, in consequence of such halocline features, salinity changes were stronger under AU simulations with water from 130 m than with water from 550 m depth in GBR and HWI, while the case turned opposite in SCS (**Figure 4**).

The model control run for area-averaged annual mean sea surface Ω-Arag (aa-Ω) predicted a decrease by more than 1.3 units for 80 years' course, reaching 2.15 (GBR), 2.17 (SCS), and 2 (HWI) in 2099. The implementation of AU decreased aa-Ω by < 0.2 units in the "*shallow_low*", "*shallow_high*" and "*deep_low*" runs, and by > 0.5 units in the "*deep_high*" run relative to the control in all three regions in 2099 (**Figure 4**). Namely, the aa-(s in year 2099 for runs "shallow_low", "shallow_high", "deep_low", and "deep_high" were: 2.15, 2.10, 2.10, and 1.48, respectively, in GBR; 2.17, 2.13, 2.15, and 1.52, respectively, in SCS; and 2.00, 1.97, 1.93, and 1.56, respectively, in

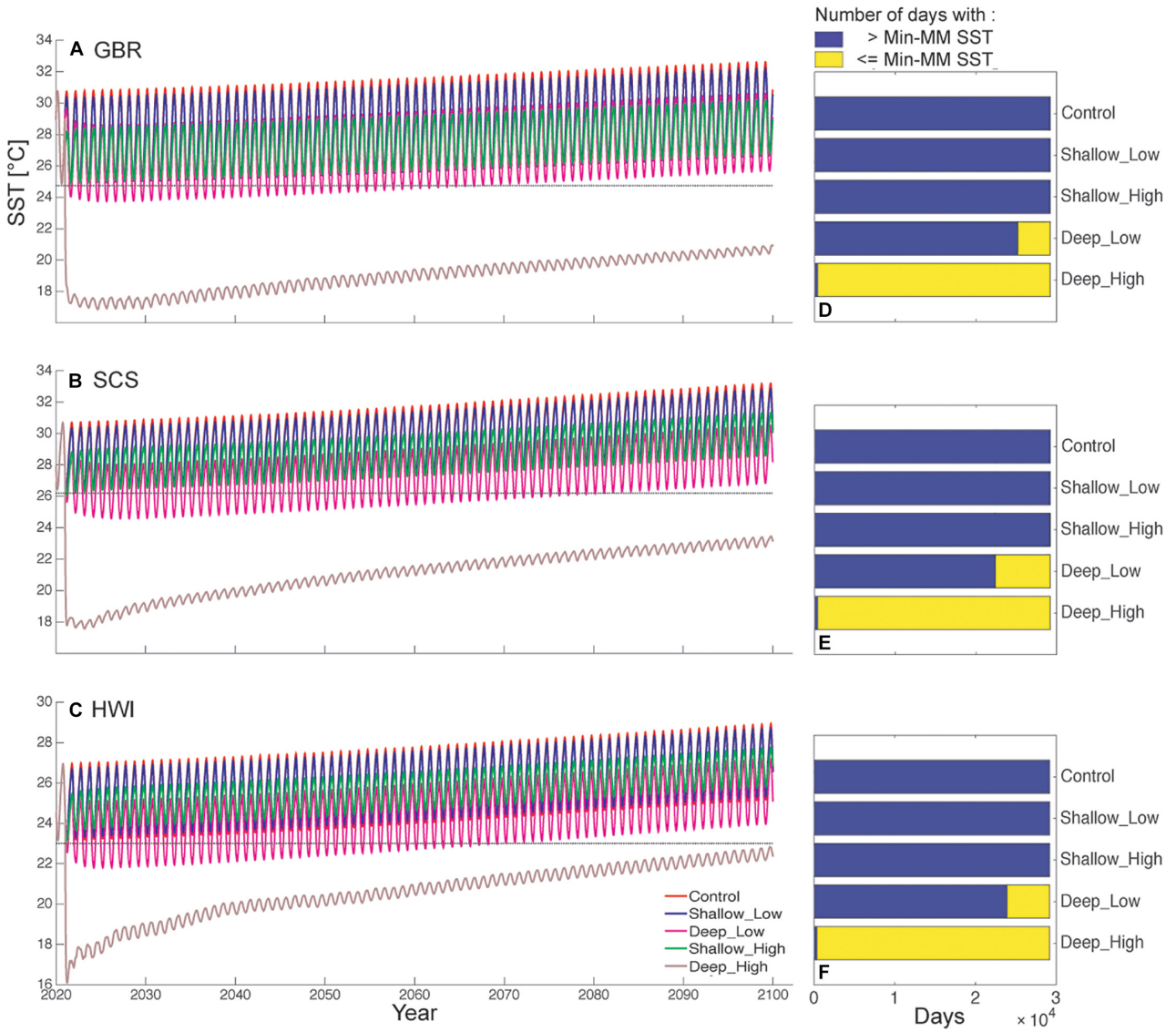

FIGURE 3 | Results of model runs for the Great Barrier Reef (GBR), South China Sea (SCS), and Hawaii (HWI) regions: The panels show area-averaged 5-day mean time series of sea surface temperature (SST) since 2020 **(A–C)**, and accumulated number of days with area-averaged SST below the coldest monthly mean climatology (Min-MM) by the end of 2099 **(D–F)**. Coldest monthly mean climatology SST was highlighted with black lines for each region in panel **(A–C)**.

HWI. Water from the deeper ocean layer in our test regions had lower (-Arag than the surface water (**Supplementary Figure S5**), which explains why the surface (-Arag decreased near AU sites.

The model control run for area-averaged annual mean sea surface nitrate concentration (aa-N) decreased by < 0.1 μmol/l in 80 years, reaching 0.157 (GBR), 0.218 (SCS), and 0.058 μmol/l (HWI) in 2099. Due to a higher nitrate concentration in the deep compared to the shallow, the AU model runs "*shallow_low*", "*shallow_high*", "*deep_low*", and "*deep_high*" increased aa-N to 0.182, 0.315, 0.563, and 18.4 μmol/l, respectively, in GBR; 0.122, 0.127, 0.892, and 19.9 μmol/l, respectively, in SCS; and 0.058, 0.059, 0.398, and 11.2 μmol/l, respectively, in HWI (**Figure 4**). The magnitudes of the perturbations in those three variables from "*shallow_low,*" "*shallow_high,*" and "*deep_low*" were well within the seasonal variability for local inorganic nitrate. The "*deep_high*" run, however, increased surface nutrient concentration by two orders of magnitude, which was far beyond natural nutrient fluctuations (**Supplementary Figures S6–S8**).

Corals, along with other marine biota, can be sensitive to changes in oceanic salinity, Ω-Arag and nutrient levels, and AU could impose harmful impacts on ecosystems if changes of these parameters are too strong. With respect to concomitant occurrences of salinity changes and temperature increase as shown by our result e.g., from "*deep_high,*" the threat of thermal stress likely surpasses the stress imposed by salinity anomalies in corals (Buddemeier and Fautin, 1993; Reynaud et al., 2004; Chavanich et al., 2009). AU-induced changes in salinity are relatively small (<1PSU) and may therefore pose a minimal risk

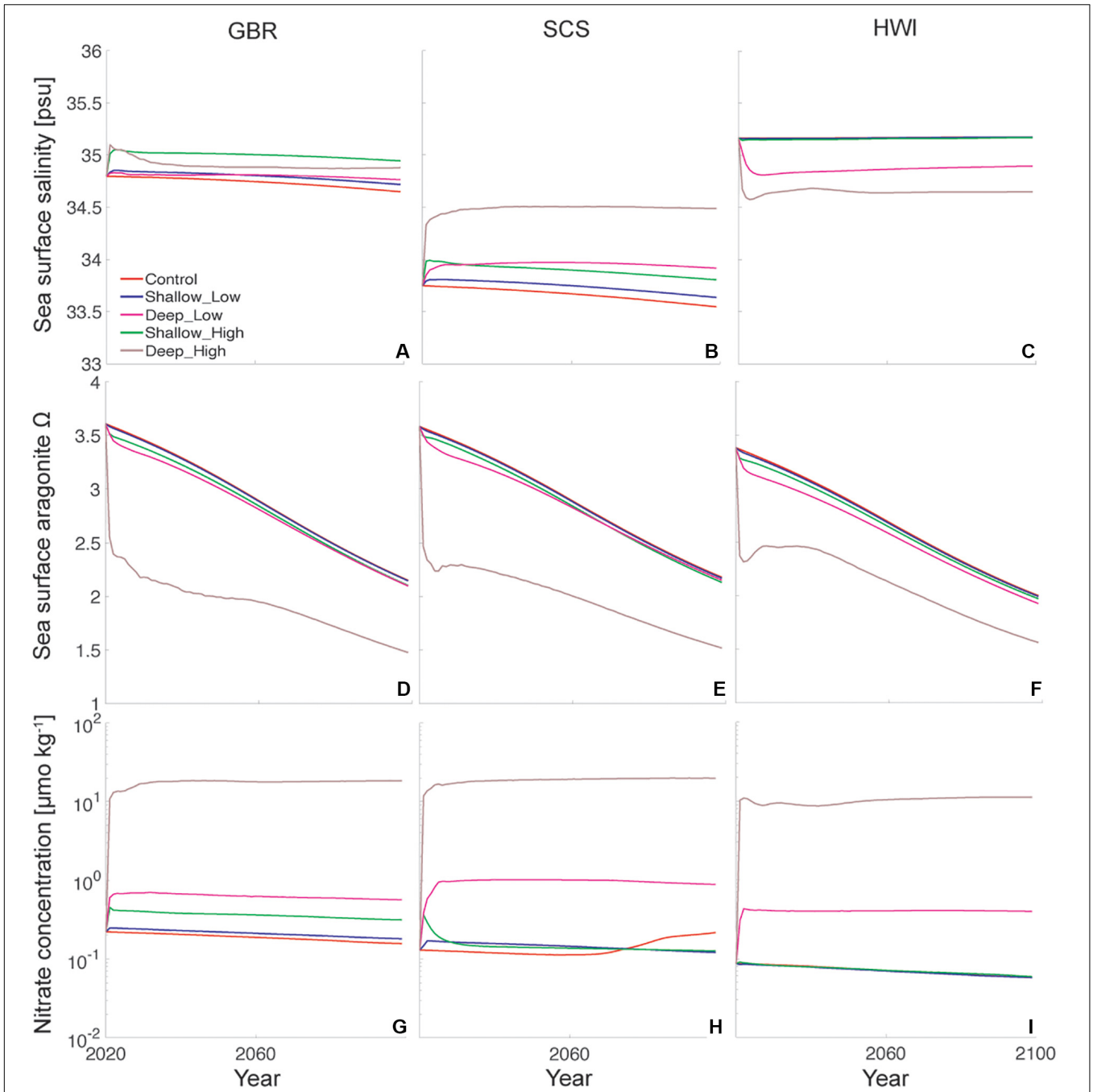

FIGURE 4 | For the Great Barrier Reef (GBR), South China Sea (SCS), and Hawaii (HWI) regions, the time series of area-averaged annual mean sea surface salinity **(A–C)**, aragonite saturation level (Ω-Arag, **D–F**), and nitrate concentration **(G–I)**, where artificial upwelling was implemented.

for corals (Hoegh-Guldberg and Smith, 1989; Humphrey et al., 2008). However, these changes in salinity may have a stronger effect on water density distribution and hence flow patterns. Along with thermal stress, ocean acidification (i.e., reductions in Ω-Arag), driven by progressing climate change, is considered a major global challenge to coral reefs and other marine ecosystems (Orr et al., 2005). This is because reductions in Ω-Arag suppresses calcification in corals and other marine calcifiers and thereby weaken their skeleton or other stabilizing structures (Gattuso et al., 1998; Ricke et al., 2013; Wittmann and Poertner, 2013). The decline in Ω-Arag of up to 1.3 units over the next 80 years (as demonstrated in the model control run) could transgress the Ω-Arag safe level of 2.82 for corals (Guan et al., 2015) and is therefore a major concern for the persistence of coral reefs (Orr et al., 2005; Hoegh-Guldberg et al., 2007). The further declines of Ω-Arag caused in particular by rather extreme AU scenarios

(e.g., "*deep_high*" scenario) will intensify ocean acidification thus likely making environments inhabitable to corals (Kleypas et al., 1999). Moderate increases in inorganic nutrient supply (e.g., nitrate) may potentially lower the negative effects of ocean acidification, by increasing phytoplankton growth and hence the availability of energy (through feeding) for increased investment into calcification (Koop et al., 2001; Bongiorni et al., 2003). Increased heterotrophy in corals through increased food supply has previously been hypothesized to be the reason for increasing coral calcification despite decreasing Ω-Arag in Bermuda over the past two decades (Bates, 2017). Increased inorganic nutrient supply in general is known to either be neutral or beneficial for corals, since they can promote zooxanthellae growth and hence primary production and energy supply to corals (Fabricius, 2005). Negative effects, however, may still occur under extreme nutrient enrichment (e.g., "*deep_high*" AU scenario), where nitrate concentrations are well above the suggested upper threshold for coral reefs of 4.51 μmol/l (Guan et al., 2015). Negative effects on organism (coral) level can include (*i*) a loss of control of the coral host over the zooxanthellae population (Falkowski et al., 1993), and *(ii)* increased susceptibility to coral diseases and bleaching (Vega Thurber et al., 2013) in particular, if nutrient supply is unbalanced (N:P ratio; Wiedenmann et al., 2012). Negative effects on ecosystem level include, for example, *(i)* hypoxic conditions during mass die-off of algal biomass (Gray et al., 2002; Keller et al., 2014), and *(ii)* shifts in benthic community structures toward algae dominated ecosystems in cases of low herbivore abundance (e.g., due to overfishing,; McCook, 1999; Szmant, 2002). Therefore, we conclude that AU implementation causing a moderate inorganic nutrient enrichment of surface waters, represented by cases of "*shallow_low,*" "*shallow_high,*" and "*deep_low*" (below 4.51 μmol/l) might be tolerable or even beneficial for coral reefs, while AU implementations causing strong enrichment in coral reefs and beyond shown by "*deep_high*" is likely damaging and should therefore be avoided.

Implications for Real AU Practices

Our modeling results provide valuable information on the potential benefits and risks of AU on coral reefs and beyond. It shows that SST and thermal stress can be effectively reduced by AU, which, based on the reduction of DHW, either fully prevented heat-induced coral bleaching until the year 2099 ("*shallow_high,*" "*deep_low,*" and "*deep_high*" modes), or delayed the occurrence of bleaching and reduced its severity at a low upwelling intensity ("*shallow_low*"). In the "*deep_high*" run, AU induced perturbations that profoundly reshaped the hydrology and biogeochemistry, and triggered changes that were not seen in the preceding decades. Therefore, the AU settings in this model run are not practical. In terms of lowering coral bleaching threat, the "*shallow_high*" and "*deep_low*" runs produced similar results as the "*deep_high*" run, but with reduced adverse environmental impacts caused by temperature drops, salinity changes, Ω-Arag reductions and nutrient enrichment. These results imply that low to moderate upwelling rates and rather shallow upwelling depths are more practical to reduce undesirable effects while maintaining the efficiency of bleaching mitigation. Although "*shallow_low*" did not completely prevent bleaching for the entire duration of 80 years, it could effectively prevent BL_LV1 conditions before the year 2045 in SCS (**Supplementary Figure S4**). The delay in bleaching conditions caused by "*shallow_low*" run would thereby at least provide temporal refugia in which corals may adjust to increasing SST or in which other adaption technologies are developed. With respect to the temporal scale, the modeling results suggest that AU intensity should be adjusted over time following the predicted increase in SST (Meinshausen et al., 2011) and a predicted increase in the frequency of ENSO events entailing heat waves (Cai et al., 2014). Thus, AU intensity would need to be increased over time either by increasing the depth or the volume of upwelled water. At the same time, coral bleaching events usually occur in only abnormally warm summers, for several days to weeks. Therefore, AU may only be necessary for a given period of time when thermal threats occur. The continuous operation of AU runs as applied in our study, provides unnecessary upwelling throughout a large portion of the year, which most likely substantially exacerbating the overall negative side effects. Concerning the complexities in temporal patterns of the SST and heat stress under climate change, the ideal AU operation needs to be firmly guided by timely monitored and forecasted temperature profiles at targeted areas.

Although not tested in this study due to limitations of the Earth system model, another possible pathway to reduce the strong environmental impact seen in the "*deep_high*" run would be to reduce the geographical density of AU or to limit AU to locations close to the reef only. Indeed, the uniformly deployed arrays of AU water translocation pathways in the model covered large areas that were not inhabited by corals (**Supplementary Figure S2**). Such an approach will be difficult and undesirable to apply in practice, because of space constraints over the ocean and the enormous engineering costs (Matsuda et al., 1999; Kirke, 2003). At GBR, for example, coral reefs occurred only in less than 10% of the deployment area, while the excess of AU devices certainly contributed to the unintended environmental perturbations locally and elsewhere. It is more likely that the availability of the AU hardware, technology, and funds will allow very localized AU implementations, which will protect only a small fraction of reefs. Thus, if the AU pipes are deployed only at and near the reef sites, we might expect much lower overall environmental changes over the whole area even under intense upwelling scenario ("*deep_high*").

The choice of the AU settings is not only a question of ecological benefits and risks, but also what is feasible from an engineering point of view in installation and operation. Advanced AU devices with stronger turbine, higher working efficiency and adiabatic pipelines could pump enough cold water to successfully prevent coral bleaching over larger areas, but they are very costly (Matsuda et al., 1999). Upwelling seawater from a depth beyond our proposed AU devices (from 130 m or 550 m Liu and Jin, 1995; Kirke, 2003;, see **Supplementary Text S3** for technical details) is theoretically possible, however extending the upwelling pipe to deeper levels, will make the construction and maintenance of the devices more difficult (Kirke, 2003). In addition, many reef flats can extend to hundred kilometers horizontally without evidently stratified vertical sea temperature.

In this situation, the installation of pipelines that connects an outlying deep water source, with coral living habitat might be the most expensive investment. When it comes to pumping rates we also need to consider energy demands and balance AU settings with its bleaching mitigation potential. In our rough estimation (**Figure 5**), increasing the AU vertical depth increases the energy demand to pump 1 m^3 bottom water to sea surface almost linearly. However, increasing AU rates can increase the energy demand to upwell the same amount of water to the surface at a much lower rate (**Figure 5**). Therefore, for AU settings with similar cooling effects as demonstrated for "*shallow_high*" and "*deep_low*" runs, the "*shallow_high*" should be preferred for energy-saving causes (see **Supplementary Text S4** for detailed calculations). Overall, the bleaching mitigation effectiveness and the costs of AU devices are both strongly determined by local circumstances and need careful balancing of benefits, risks and trade-offs.

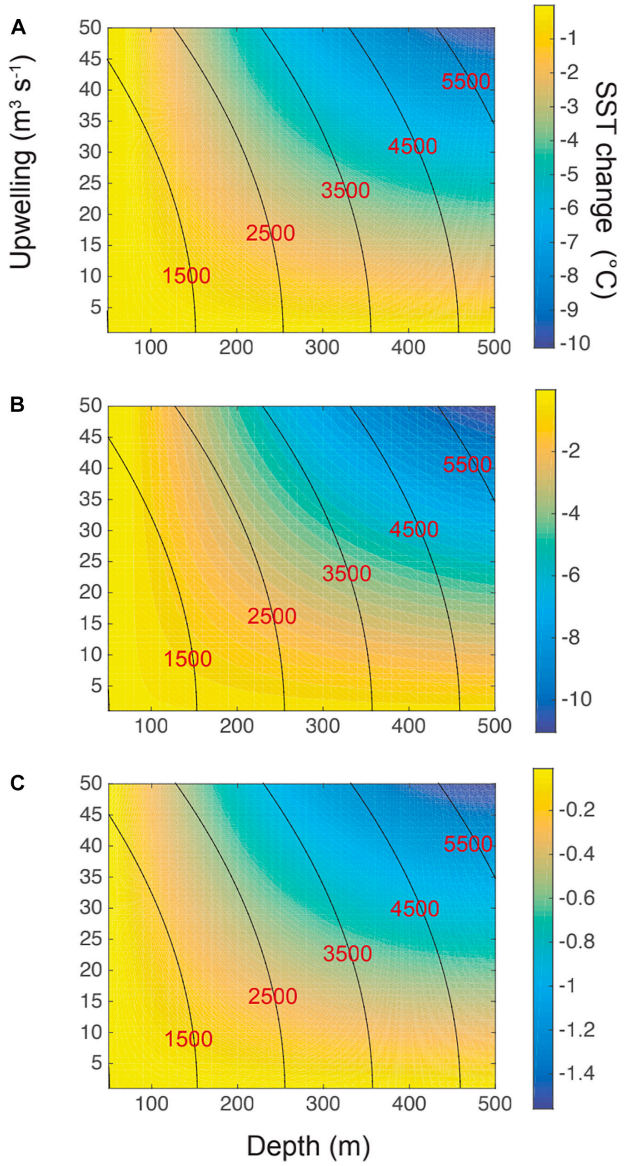

FIGURE 5 | The quantitative analysis for required AU input by investigating the surface cooling effects after the first-year's AU implementation. Area-mean SST differences between AU runs and control run are plotted against AU upwelling rate and AU depth for regions GBR **(A)**, SCS **(B)**, and HWI **(C)**. To pump 1 m^3 bottom water to sea surface adiabatically, required energy (unit: kJ) is plotted as isoclines over the SST fields.

CONCLUSION

Monitored heat stress and predicted future global warming have prompted the search for ways to protect coral reefs from severe bleaching. In this context, we have assessed the environmental impacts of virtually implemented water translocation, as an analogous process to AU, over large areas that included coral reefs with an Earth System modeling approach. We used constant upwelling rates over 80 years for 500 km^2 regions. Although these scenarios are beyond of what would be temporally and spatially feasible and desirable *in situ*, the model results provide valuable information about the effect of AU on the perturbations of ocean heat and biogeochemistry. The results clearly show that AU has the potential to effectively reduce SST and DHW, and delay the occurrence of future coral bleaching events. However, the potential side effects of AU, such as surface overcooling, salinity fluctuations, surface acidification, and nutrient enrichment, can cause severe risks to corals, in particular, if water is upwelled from deep layer (e.g., 550 m) at high rates. Though it was not discussed in detail, model simulations also revealed changes of ocean circulation under the deployment of more severe AU scenarios, which can cause unexpected perturbations in ocean heat budget and biogeochemistry (**Supplementary Text S5**). In summary, our model evaluation indicates that even rather low upwelling rates of water derived from rather shallow depth (here 130 m) can be an effective measure to prevent coral bleaching during thermal stress, while at the same time minimizing undesirable environmental side effects.

In order to explore this topic further and to validate and refine our model results, future studies require regional models with a higher spatial and temporal resolution as well as experimental work. Although the development of regional models that allow simulations of AU scenarios is challenging, they are necessary to provide region-specific details about the biogeochemical and hydrographic consequences of AU, which may be strongly influenced by the local bathymetry and hydrographic conditions. In addition, experimental studies that investigate the effectiveness of different AU scenarios on different coral species in different regions to guide the selection of AU sites and operating strategies, are necessary. In this regard, performing a medium to long-term experiment with focus on analyzing heat-stressed corals' physiological response to cool subthermocline water analogous to AU, can be a meaningful attempt. This can provide important insights for how AU can be operated to provide stress relief while reducing side effects (Sawall et al., 2020), and suggest further refined set-ups for modeling studies. Current technology would theoretically allow successful installation and operation of AU devices locally in shallow coral reef environment. The biggest

technological challenge, however, is likely the accessibility of cool water. With oceanic deep water being distant to many shallow water corals, a possible solution to such natural constraint could be exploiting underground cool brine water from aquifer layers. Its effectiveness and potential risks, however, remain to be investigated. If climate change follows a business-as-usual scenario in the future and AU technology proves to be useful to mitigate heating stress, AU can probably buy corals some time, required that adequate funds, human power, infrastructure, and space are available. Although in the future the obstacles to the implementation of AU and its environmental side effects may be overcome with the development of new AU hardware and wisely planned preparations, it is still necessary to limit climate change to well below the 1.5°C warming, since human interventions will not be able to save coral reefs on a global scale.

DATA AVAILABILITY STATEMENT

Datasets (model simulations and computer codes to generate figures/tables) for this research are available in GEOMAR repository (https://ftp3.geomar.de/users/yfeng/CoralAU/).

AUTHOR CONTRIBUTIONS

EF initiated the idea of the study, conducted the study, performed data analysis, and wrote the manuscript. YS, MW, ML, and YF contributed conception and design of the study. All authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Sea Urchins Play an Increasingly Important Role for Coral Resilience Across Reefs in Taiwan

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Herbivores are an important functional group that control algae, create new space, and promote recruitment for coral recovery. However, on many coral reefs, overfishing has greatly decreased the density of herbivores, especially fishes and gastropods, impairing coral resilience. On such overfished reefs, remnant herbivores that are not target species of local fisheries, e.g., sea urchins, are expected to play an increasingly important role, yet few studies, except for those in the Caribbean and Kenya have examined non-fish herbivores in relation to coral resilience. Here, we conducted field surveys at 30 sites along three coral reefs in Taiwan between 2016 and 2017, to examine the relative importance of six key factors for coral resilience: herbivore abundance (fishes, gastropods, sea urchins), coral cover, macroalgal cover, habitat complexity, water depth, and wave exposure. The density of juvenile coral was used as a proxy of coral resilience. Diadematid sea urchins (*Echinothrix* spp. and *Diadema* spp.) dominated most sites (19 of 30 sites) and multivariable regression models showed sea urchin density as the best positive predictor of coral juvenile density. The results elucidated the increasing role diadematid sea urchins play as remnant herbivores on overfished coral reefs in Taiwan. Given that overfishing is a widespread issue, this phenomenon may be occurring globally. More studies are needed to examine the role of remnant, but often ignored, sea urchin herbivory on coral resilience. Reef managers should consider monitoring locally remnant herbivores and incorporating them into management strategies.

Keywords: overfishing, herbivore, sea urchin, coral recruitment, Taiwan, Southeast Asia

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INTRODUCTION

Coral reefs are in global decline due to chronic anthropogenic disturbances (Pandolfi et al., 2003; Bellwood et al., 2004; Hoegh-Guldberg et al., 2007; Hughes et al., 2018). To reverse these trends, resilience-based management has been proposed to enhance the recovery potential of coral reefs (Bellwood et al., 2004; Hughes et al., 2017). Resilience-based management requires the control of current anthropogenic stressors and to encourage the recovery process (Mcleod et al., 2019). However, information on factors and conditions that promote recovery of coral reef is still scarce (Hughes et al., 2010).

Resilience studies are commonly based on long-term monitoring of recovery process seen in target populations/communities (Graham et al., 2015). This time-consuming process becomes a bottleneck in the advancement of resilience studies, particularly in slower dynamic communities such as coral reefs. The density of juvenile corals has been suggested as a good indicator to assess the resilience of coral populations (hereafter coral resilience) (Gilmour et al., 2013; Graham et al., 2015; Dajka et al., 2019; Nozawa et al., 2020). This is because coral recovery is typically initiated by recruits (Hughes et al., 2007b; Gilmour et al., 2013; Graham et al., 2015), and a positive correlation between juvenile coral density and recovery of coral populations has often been observed (Gilmour et al., 2013; Dajka et al., 2019; Nozawa et al., 2020). This new approach to evaluate coral resilience based on juvenile coral density significantly shortens study time and allows scientists to explore factors influencing coral resilience more efficiently.

Coral resilience has been explained by a hypothesis concerning the quantitative interactions between three key functional groups: herbivores, algae, and corals (Bellwood et al., 2004; Hughes et al., 2007a; Mumby et al., 2007; Graham et al., 2013). In a healthy coral reef ecosystem, herbivores and oligotrophic water control algal abundance, the main competitor of corals for space. However, along many contemporary coral reefs, overfishing and eutrophication weakens this balance, resulting in algal dominance on reef space that hinders coral recovery, via the coral recruitment process (Birrell et al., 2008; Mumby and Steneck, 2008). Therefore, the abundance of herbivores and algae has been considered as the main biotic factor determining coral resilience (Bellwood et al., 2004; Mumby et al., 2006).

Although less attention is paid to it, the abundance of coral itself could also influence coral resilience through stock-recruitment dynamics (Hughes et al., 2019) and competition for space between adults and recruits (Ledlie et al., 2007). Coral abundance determines the amount of locally produced larvae, which contributes to self-recruitment, especially for coral species with larvae of short-distance dispersal, such as *Acropora* spp. (Nozawa and Harrison, 2008) and *Pocillopora* spp. (Tioho et al., 2001). Coral abundance also determines the degree of competition for space between adult corals and recruits, thereby influencing coral recruit density (Baird and Hughes, 2000).

Besides the biotic factors, three abiotic factors have been proposed to influence coral resilience via the herbivore-alga-coral interaction: habitat complexity, water depth, and wave exposure (Mumby and Steneck, 2008; McClanahan and Muthiga, 2016). Habitat complexity influences the abundance of herbivorous fish with more complex habitats harboring increased herbivore fish abundance, while water depth covaries with light intensity, directly affecting algal abundance (Ledlie et al., 2007; Graham et al., 2015). Graham et al. (2015) highlighted habitat complexity and water depth as two key factors of coral resilience, among others, over a 17-year coral recovery documented in the Seychelles. Wave exposure is less examined in coral resilience studies, but known to influence the abundance of herbivores (Harborne et al., 2006; Bronstein and Loya, 2014) as well as the abundance and taxonomic composition of corals (Robinson et al., 2019).

To date, only a few comprehensive studies have been conducted to examine the relative importance of key factors proposed for coral resilience (Graham et al., 2015; Dajka et al., 2019; Mcleod et al., 2019). Furthermore, previous studies of coral resilience have mainly been conducted in the Caribbean (Carpenter and Edmunds, 2006; Furman and Heck, 2009; Idjadi et al., 2010), Great Barrier Reef (Darling et al., 2017), and the Seychelles (O'Leary et al., 2013; Graham et al., 2015; Dajka et al., 2019), with few studies from Southeast Asia (Muallil et al., 2020; Nozawa et al., 2020). In the present study, we examined the relative importance of herbivore and five other key factors for coral resilience: algae, corals, habitat complexity, water depth, and wave exposure at a total of 30 sites along three Taiwanese coral reefs, using juvenile coral density as an indicator of coral resilience. We focused on herbivores by covering both herbivorous fishes and benthic herbivores (sea urchins and gastropods).

MATERIALS AND METHODS

Field surveys were conducted along three coral reefs in southern Taiwan between 2016 and 2017 (**Figure 1**). At each coral reef, 9–11 sites were haphazardly selected at 4–13 m depth (**Figure 1**). A total of 30 sites were investigated, including 10 sites at Green Island, 11 sites at Orchid Island, and 9 sites in Kenting. At each site, we collected data within an area approximately equal to 50 m × 50 m.

In this study, we investigated the density of juvenile corals (<5 cm in diameter) as a proxy for coral resilience and six

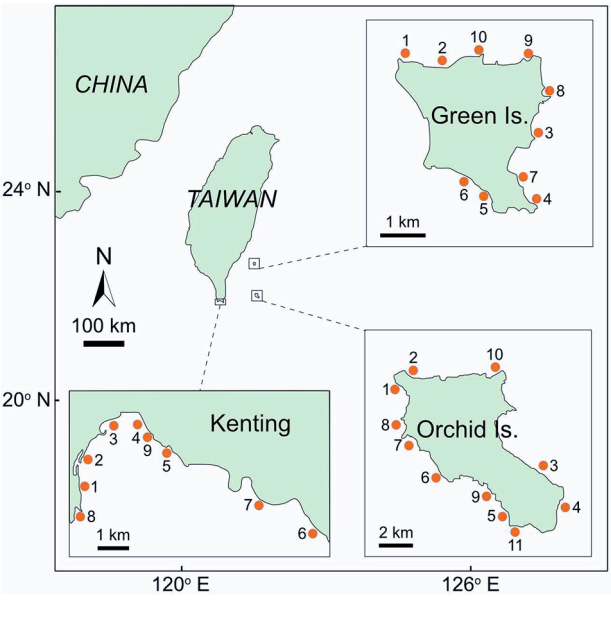

FIGURE 1 | Survey sites (indicated by point) in three coral reef areas in southern Taiwan: Green Island, Orchid Island, and Kenting. The ID number of survey sites at each coral reef corresponds to **Supplementary Table S1**, where a summary of the results for each site is provided.

factors that would influence juvenile coral densities: corals, macroalgae, herbivores, habitat complexity, water depth, and wave exposure. For herbivores, density of herbivorous fish (parrot and surgeonfish), sea urchins, and herbivorous gastropods were recorded. Habitat complexity was examined at two size-scales (decimeter and meter scale). Herbivorous fish, habitat complexity (m-scale), and wave exposure were measured at each site, while the other data were collected along a 10-m line transect (n = 5) at each site (**Supplementary Figure S1**). Haphazard sampling was frequently used when the spatial distribution of coral communities was unknown and random sampling raised implementational problems. Although haphazard sampling is common in field ecological studies, we acknowledge that statistical tests are not strictly valid and results could be more vulnerable to unmeasurable bias (Lewis, 2004).

Coral and macroalgal cover were estimated using the line intercept method. Five replicates of a 10-m line were placed haphazardly along hard substrate at each site (**Supplementary Figure S1**). Corals and macroalgae (>2 cm in trunk height) appearing below the line were photographed from above with the line. Close-up photographs were also taken for taxonomic identification to the genus level (Veron and Stafford-Smith, 2000; Wang et al., 2015). The sum of intercept distance of corals or macroalgae on each line transect was measured on photographs to determine coverage (%) as a proportion of tape length.

The density of benthic herbivores (sea urchins and herbivorous gastropods) were estimated within five replicate 10 × 2 m belt transects (**Supplementary Figure S1**). The number of sea urchins and macro-gastropods (>1 cm) appearing within a 1-m distance from the 10-m line-transect within both the left and right-hand sides were counted by a scuba diver using a 1-m scale as a reference. Observed individuals were photographed with a scale for taxonomic identification. At the study sites, sea urchins and gastropods were hiding in holes and crevices during the daytime survey. Therefore, care was taken to detect individuals by spending time (>10 min per survey area) looking closely into habitats. We focused on free-grazing sea urchins and ignored taxa that exclusively inhabit holes and burrows in the study locations, including *Echinostrephus* spp. and *Echinometra* spp.

The density of juvenile corals (<5 cm in diameter) was examined using 50 cm × 50 cm quadrats (n = 5) placed haphazardly on hard substrata along the 10-m line transect (**Supplementary Figure S1**). Quadrats were placed on relatively vacant space where >50% of the quadrat area was not occupied by sessile macro-benthos (e.g., hard corals, soft corals, macroalgae, sponges, and sea anemones). Juvenile corals in each quadrat were counted and photographed with a scale for taxonomic identification to the genus level (Veron and Stafford-Smith, 2000). The majority of juvenile corals that were larger than ∼1 cm in diameter could be taxonomically identified. The density of juvenile corals (m^{-2}) was calculated by dividing the sum of juveniles by the amount of non-occupied area by the sessile macro-benthos in the five quadrants. The non-occupied area in each quadrat was estimated by using CPCe (Coral Point Count with Excel Extensions, ver. 4.0 software) with 100 random points (Kohler and Gill, 2006).

The abundance of herbivorous fish was estimated using the modified method of stationary point count (Colvocoresses and Acosta, 2007). Parrotfish and surgeonfish were surveyed as regionally dominant herbivorous fish groups (Muallil et al., 2020; Nozawa et al., 2020). Although we assumed both parrotfish and surgeonfish are herbivorous in this study, caution is advised when interpreting results as recent studies have indicated that many taxa in such groups are not entirely herbivores and instead feed mainly on cyanobacteria, microbes, and detritus (Clements et al., 2017; Dell et al., 2020). In the survey, three 360-degree video cameras (PIXPRO SP360, Kodak) were placed haphazardly (∼10 m away from each transect line) within the 50 m × 50 m area at each site. Each video camera was set at the time-lapse mode with an interval of 1 s with an image quality of 1440 × 1440 pixels. Videos were taken for approximately 40 min, while conducting other surveys at each site. Preliminary examination on the resolution of the video camera indicated that images of target fish taxa that are larger than 10 cm in total length could be identified within the radius of 5 m from the 360-degree video camera (ca. 78.5 m^2). Therefore, we used these numbers for the abundance estimation. Due to the small survey area of each video camera, we estimated the density of herbivorous fishes at each site using the sum of fishes (>10 cm in total length) recorded by the three video cameras (total survey area of ca. 235 m^2).

Habitat complexity was assessed at 2 size-scales: decimeter-scale (relief of <1 m) and meter-scale (relief of >1 m). Habitat complexity at the dm-scale was measured based on the undulation of substrate surface along the 10-m transect line using a water level logger (HOBO Water Level Data Logger; Onset Computer Corporation, Bourne, MA, United States) following Dustan et al. (2013). The water level logger also recorded depth, and average depth was calculated for each line and used in the analysis. The rugosity index was calculated by dividing the measured length of surface undulation by the measured horizontal distance (10 m) (Graham and Nash, 2013). For m-scale complexity, the seascape complexity was determined by visual estimation using four categories defined based on the abundance of large relief (>1-m height) and deep crevices (>1-m deep) at the study site: category 1 = few (abundance < 3); category 2 = several (abundance of 3–10); category 3 = many and moderately complex (abundance of >10); category 4 = very complex (abundance of >10, the max size of relief and crevices is much larger than the category 3).

Wave exposure at each site was estimated by the fetch method (Chollett and Mumby, 2012). The fetch was calculated using the fetchR package in R (R Core Team, 2018) with 36 compass directions (angle intervals of 10°). Data on wind speed and direction (2007–2018) at each site were collected from the online archive, CODiS of Taiwan Central Weather Bureau[1]. Wave exposure at each site (J m^{-3}) was calculated based on these values using the linear wave theory that includes equations of "fetch-limited" and "fully developed" seas (Ekebom et al., 2003; Harborne et al., 2006; Chollett and Mumby, 2012).

We examined factors that would influence the density of juvenile corals using generalized linear mixed models (GLMMs).

[1]http://eservice.cwb.gov.tw/HistoryDataQuery/index.jsp

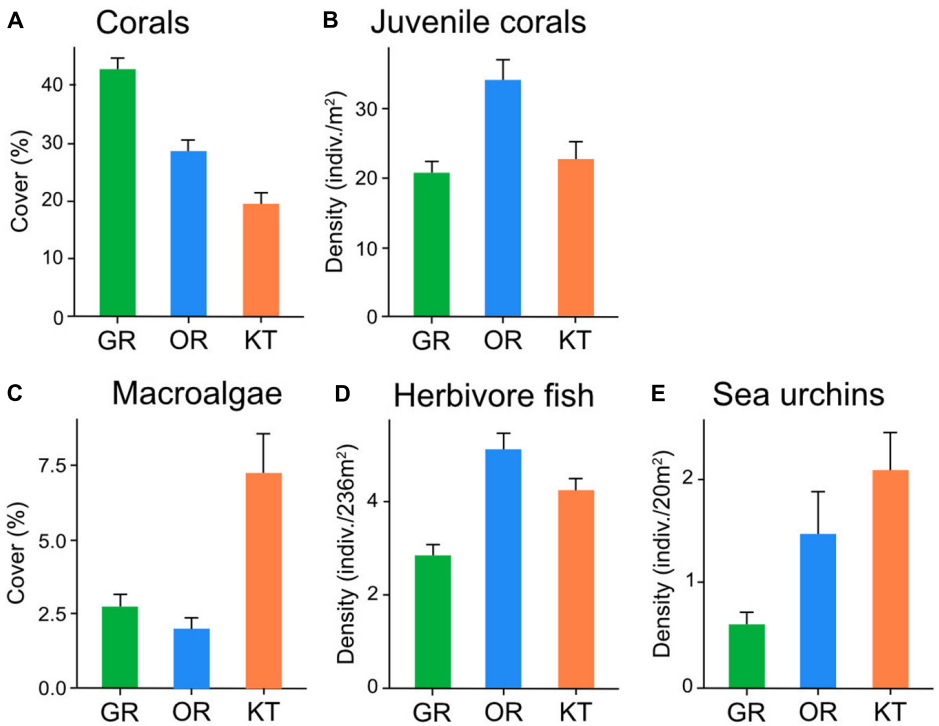

FIGURE 2 | Abundance [mean ± SE] of **(A)** hard coral, **(B)** juvenile coral, **(C)** macroalgae, **(D)** herbivorous fish, **(E)** sea urchins (mostly diadematids) on three Taiwanese coral reefs in 2016–2017. The densities of juvenile coral, sea urchins, and herbivorous fish have different scales.

A Gamma error distribution with a log link function was used for the response variable (juvenile coral density). In the model, eight predictor variables were considered: herbivorous fish density, sea urchin density, coral cover, macroalgal cover, dm-scale habitat complexity, m-scale habitat complexity, water depth, and wave exposure. Herbivorous gastropods were virtually absent at most sites and therefore ignored in the analysis (see "Results" section). Juvenile sea urchins (<2 cm in test size) are less influential in controlling algae and consequently were excluded from sea urchin density data. In the analysis, predictor variables were standardized by subtracting the mean and dividing by two standard deviations (Gelman, 2008). We first examined an overall model by combining all data from the three coral reef areas and then examining individual models for each coral reef area. Study sites that were nested within coral reef areas were treated as random factors. No multicollinearity was detected in the models at a criterion of variance inflation factors < 2.5. The Akaike information criterion corrected for small size (AIC_c) was used for model selection. Best models of <2 ΔAIC_c were selected, and model averaging was performed within them (Burnham and Anderson, 2002; Zuur et al., 2009). In the averaged model, the mean and 95% confidence intervals (CIs) of the estimated coefficients were calculated for each predictor variable. Predictors were inferred as significant when 95% CIs of coefficient excluded zero. The relative importance of each predictor in the averaged model was calculated by summing the Akaike weights for the predictor over the best models (Burnham and Anderson, 2002). This index ranges from 0 to 1 with a higher value for the higher relative importance. All analyses were performed in R ver. 3.6.1 (R Core Team, 2018) with the packages, lme4 ver. 1.1-23 and MuMin ver. 1.43.10.

RESULTS

We described the state of the six biological variables: corals, juvenile corals, herbivorous fishes, sea urchins, herbivorous gastropods, and macroalgae recorded along the three Taiwanese coral reefs. A summary of results at each site for the biotic variables and three abiotic variables (habitat complexity, water depth, wave exposure) is provided in **Supplementary Table S1**.

Coral cover varied among the three reefs with the highest average cover recorded in Green Island [mean ± SE: 42.9 ± 1.8%], followed by Orchid Island [28.4 ± 1.9%], and Kenting [19.4 ± 1.7%] (**Figure 2A**). Of the total 50 coral genera identified, those dominant in total cover were *Montipora* (23.7%), *Porites* (16.9%), *Pocillopora* (8.4%), and *Acropora* (8%). In contrast, the density of juvenile coral was the highest in Orchid Island [mean ± SE: 34 ± 2.6 indiv./m^2], followed by Kenting [22.7 ± 2.4 indiv./m^2] and Green Island [20.8 ± 1.3 indiv./m^2] (**Figure 2B**). The dominant genera of juvenile corals were similar to those of adult corals, including *Porites* (26%), *Montipora* (17%), and *Pocillopora* (14%).

Macroalgal cover was relatively low at all sites, ranging from 0 to 28.1% on average. Kenting had the highest macroalgal cover [mean ± SE: 7.4 ± 1.3%], followed by Green Island [2.8 ± 0.4%],

and Orchid Island [2.1 ± 0.3%] (**Figure 2C**). Of a total of 19 genera identified, dominant genera in total cover were *Codium* (29.3%), *Galaxaura* (21.6%), and *Laurencia* (16.1%).

The overall density of herbivorous fish (parrotfish and surgeonfish) was low [mean ± SE: 4.2 ± 0.2 indiv./235 m^2] on these Taiwanese coral reefs. Orchid Island had the highest average density [5.1 ± 0.3 indiv./235 m^2], followed by Kenting [4.3 ± 0.3 indiv./235 m^2] and Green Island [2.93 ± 0.2 indiv./235 m^2] (**Figure 2D**). Surgeonfish dominated herbivore fish assemblages (91.6%). Although taxonomic identification to genus level was difficult using the method in the present study, Nozawa et al. (2020) reported four surgeonfish genera *Acanthurus*, *Ctenochaetus*, *Naso*, and *Zebrasoma* within the coral reef areas in 2012–2014. Herbivorous gastropods were virtually absent at all sites, with only nine individuals recorded (five in Green Island, two in Orchid Island, and two in Kenting): These were *Turbo chrysostomus* (seven individuals) and *Monetaria* sp. (two individuals). Compared with herbivorous fishes and gastropods, sea urchins were the most abundant herbivores (in density) on 19 of 30 coral reef sites (**Figure 2E** and **Supplementary Table S1**). However, their density varied greatly from 0 to 14 indiv./20 m^2 among sites. The average density of sea urchins [mean ± SE] was 2 ± 0.4 indiv./20 m^2 in Kenting, 1.4 ± 0.4 indiv./20 m^2 in Orchid Island, and 0.6 ± 0.2 indiv./20 m^2 in Green Island (**Figure 2E**). Most sea urchin species belonged to the family Diadematidae, consisting of the genera *Diadema* (66.1%) and *Echinothrix* (33.9%). Except for diadematids, only three individuals of *Tripneustes gratilla* were recorded (note that *Echinostrephus* spp. and *Echinometra* spp. were not considered in this study).

Sea urchin density was the only significant positive predictor of juvenile coral density in the overall GLMM for the three Taiwanese coral reefs (coefficient = 0.415; 95% CI lower = 0.227, upper = 0.604) (**Figures 3A**, **4A**) and was also the only significant positive predictor in individual GLMMs for each reef (coefficient = 0.339–0.551) (**Figures 3B–D**, **4B–D**). The relative importance of sea urchin density was the maximum value (1.00) in all the GLMMs (**Supplementary Table S2**). Besides sea urchin density, water depth (coefficient = −0.269; 95% CI lower = −0.501, upper = −0.037) and habitat complexity at m-scale (coefficient = −0.405; 95% CI lower = −0.648, upper = −0.161) were selected as significant negative predictors in the overall GLMM for all reefs (**Figure 3A**). However, these were not significant predictors in the GLMMs for the individual reefs, except for habitat complexity at m-scale in Orchid Island (**Figure 3C**). In addition to these predictors, habitat complexity at dm-scale was selected as a significant positive predictor only in the GLMM for Green Island (**Figure 3B**).

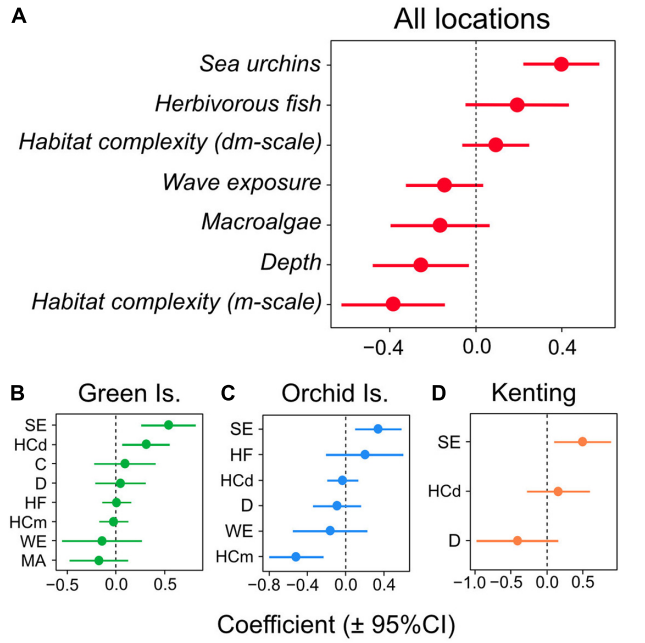

FIGURE 3 | Multi-model-averaged parameter estimates for the predictors of juvenile coral density in the multivariable regression analysis. Mean coefficient estimates (±95% confidence intervals) are plotted for **(A)** all locations, and for individual coral reefs for **(B)** Green Island, **(C)** Orchid Island, and **(D)** Kenting. Abbreviations of predictors in figures **(B–D)** denote, SE, sea urchin density; HF, herbivorous fish density; C, coral cover; MA, macroalgal cover; HCdm, habitat complexity at decimeter-scale; HCm, habitat complexity at meter-scale; D, water depth; WE, wave exposure.

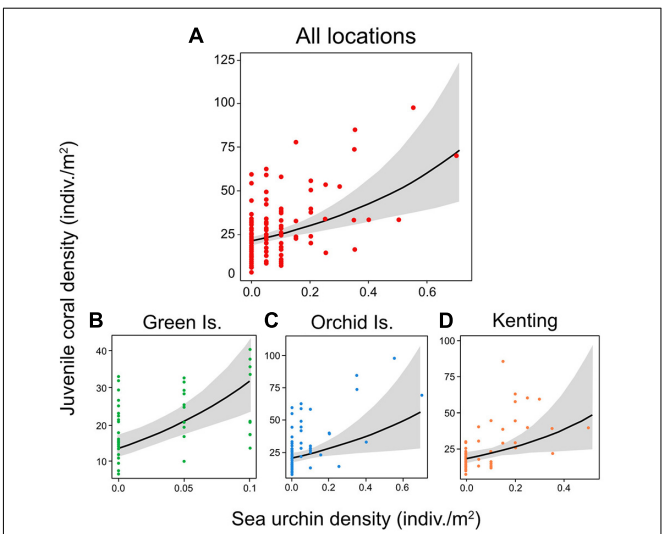

FIGURE 4 | The marginal effect of sea urchin (mostly diadematids) density (±95% confidence intervals) on juvenile coral density, estimated at the mean of other predictors in the GLMM for **(A)** all locations, and for individual coral reefs for **(B)** Green Island, **(C)** Orchid Island, and **(D)** Kenting.

DISCUSSION

The present study demonstrated that diadematid density was the primary positive driver of juvenile coral density, suggesting the important role of diadematids for coral resilience on the Taiwanese coral reefs. The dominance of diadematids was most likely owing to the population decline of other macro-herbivores, fishes and gastropods that are target species of local fisheries on coral reefs, whereas diadematids are not. In the present

study, non-diadematid sea urchins and herbivorous gastropods were rarely seen, and many herbivorous fishes were small in size (personal observation). This reflects the severe overfishing problems occurring along Taiwanese coral reefs (Dai et al., 2002) and elucidates the increasing role of diadematids as remnant herbivores on the overfished coral reefs.

A shift in dominant herbivore taxa from herbivorous fishes to sea urchins has been documented on overfished coral reefs in other regions of the world, including the Caribbean and eastern Africa (McClanahan and Kurtis, 1991; Jackson et al., 2001). Of these, the best example is *Diadema antillarum*, that became the dominant herbivore on coral reefs in the Caribbean due to historical overfishing of other herbivorous animals (Jackson et al., 2001). However, the sudden regional die-off of *D. antillarum* by an epidemic in 1983 was followed by the significant decline of coral due to overgrowth by blooms of macroalgae across the Caribbean (Lessios, 1988; Hughes, 1994). This catastrophic event revealed the important role *D. antillarum* plays as the remaining dominant herbivores pre-1983, facilitating a coral-dominated state by controlling macroalgae on the overfished Caribbean reefs (Lessios, 1988; Hughes et al., 1999; Edmunds and Carpenter, 2001; Carpenter and Edmunds, 2006). Although the recovery of *D. antillarum* populations has been prolonged and patchy (Lessios, 2016), studies have reported the strong association between their recovery, lower macroalgal abundance, and higher coral recruit abundance (Edmunds and Carpenter, 2001; Carpenter and Edmunds, 2006; Idjadi et al., 2010).

The multivariable regression analyses performed in this study indicated that only a few of the examined factors, that have been proposed as key factors for coral resilience elsewhere were significant drivers of juvenile coral abundance along Taiwanese coral reefs. The results suggest a high variation in primary factors of coral resilience among locations (Roff and Mumby, 2012) and indicates the importance of examining factors at each location. Herbivorous fishes are often dominant, regarded as the key functional group of coral resilience on pristine coral reefs (Gilmour et al., 2013) or in marine reserves (Mumby et al., 2007; Graham et al., 2015). However, on Taiwanese reefs, herbivorous fishes were not abundant, and their function was weakened (i.e., no significant correlation with juvenile coral density). Furthermore, recent studies have indicated that many nominal herbivorous fish taxa such as parrotfish and surgeonfish actually feed on non-algal foods including cyanobacteria, microbes, and detritus and are not main contributors of algal removal (Clements et al., 2017; Dell et al., 2020).

Macroalgae are also the well-known primary negative driver of coral recruits and resilience on Caribbean coral reefs (Hughes, 1994; Edmunds and Carpenter, 2001; Carpenter and Edmunds, 2006). However, outside the region, macroalgae are often not abundant and hence not a major competitor for space, as seen in the present study (Bruno et al., 2009; Roff and Mumby, 2012). Water depth was negatively associated with juvenile coral abundance in the present study, possibly owing to larval settlement patterns of the dominant coral taxa, *Porites* and *Pocillopora* spp., with more recruits at shallower sites observed locally (Nozawa et al., 2013). In contrast, it was the positive driver of coral resilience in the Seychelles (Graham et al., 2015).

Habitat complexity is a well-studied habitat characteristic that often indicates positive effects on coral recruits and resilience, owing to its positive association with herbivorous fish abundance (Graham and Nash, 2013; Graham et al., 2015). In our multivariable regression analyses, opposite effects of habitat complexity emerged depending on the habitat scale; the decimeter-scale habitat complexity showed a tendency of a positive effect on juvenile coral abundance, whereas the meter-scale habitat complexity indicated negative effects. This contrasting pattern suggests the importance of scale in habitat complexity (Tokeshi and Arakaki, 2012). It might be partially explained by the possible scale-dependent impact of habitat on grazing activity of the locally dominant herbivore, diadematids. The decimeter-scale habitat complexity could enhance diadematid abundance by providing shelter and hence, increasing gross grazing rate (Lee, 2006), whereas the meter-scale complexity would create a dynamic water environment that often reduces diadematid grazing (Foster, 1987; Rogers and Lorenzen, 2016).

A key strategy, for resilience-based management of coral reefs, is to restore functional herbivory via recovering herbivorous fish populations (Graham et al., 2013; MacNeil et al., 2015; Topor et al., 2019), while the use of benthic herbivores, including sea urchins has rarely been discussed outside the Caribbean. The use of benthic herbivores has two clear advantages over herbivorous fishes, especially for small-sized marine reserves: (1) fewer protection efforts for species like diadematids as they are often not fishery species and (2) reduced management effort because of their limited mobility. Given the time-consuming and challenging process in recovering and maintaining the sufficient density of herbivorous fish populations, including strict fishery regulations and large-sized marine reserves (MacNeil et al., 2015), it would be wise to consider benthic herbivores, ahead of, or alongside herbivorous fishes, as they may be easier to recover and manage than fish (Maciá et al., 2007; Neilson et al., 2018). However, more information is needed to realize the full potential of benthic herbivores in coral reef management. For example, grazing activity of *Diadema* sea urchins could cause damage to corals and coral recruits under high-density conditions (Sammarco, 1980, 1982; Bronstein and Loya, 2014). Research areas to fully understand the value of benthic herbivores are a priority and may include the selection of local candidate species, their effective population densities, and their effects on the alga-coral interaction.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

VD and YN conceived and designed the study, collected and analyzed the data, and drafted the manuscript. P-YC collected

and analyzed the data. C-LF, J-HS, and C-HL collected the data. AM collected the data and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Variability in Fitness Trade-Offs Amongst Coral Juveniles With Mixed Genetic Backgrounds Held in the Wild

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Novel restoration methods are currently under consideration worldwide to help coral reefs recover or become more resilient to higher temperature stress. Critical field-based information concerning the paradigm of "local is best" is lacking for many methods; information which is essential to determine the risk and feasibility associated with restoration. One method involves breeding corals from different reef regions with expected variation in heat tolerance and moving those offspring to new locations to enhance offspring survival; thereby augmenting local stock to enhance survival for anticipated warming. In this study, surviving colonies from the 2016 to 2017 mass bleaching events on the Great Barrier Reef (GBR) were reproductively crossed and they included colonies sourced from northern (three) and central (two) reefs. The gravid colonies of *Acropora tenuis* were collected across 6° of latitude, and they were spawned to produce a total of 17 purebred and hybrid crosses. Juvenile corals (3,748 individual colonies settled on 1,474 terracotta tiles) were deployed to Davies reef in the central GBR after 4 months of aquarium rearing. Survival, growth, and coral colour (as a proxy for bleaching) were assessed after 0, 91, and 217 days of field deployment. Overall, a high percentage of juveniles (17% ± 2.5 SE) survived relative to expected survival at the final census. Survival was significantly higher for central purebred crosses, hybrid crosses had intermediate survival while northern purebreds had the lowest survival. Colour and growth rates (0.001−0.006 mm^2 day^{-1}) were not significantly different amongst central, northern, or hybrid crosses but were of a reverse pattern compared to survival. On average, northern purebred crosses grew the fastest, followed by hybrid crosses, and then central purebred crosses. Modelled growth trajectories suggest that northern purebreds would take 8 years to grow to reproductive size, hybrids would take nine, and central purebreds would require 12. All deployed juvenile corals paled over time in the field although the colour of *A. tenuis* juveniles did not differ significantly amongst central, northern, or hybrid crosses. Growth and survival trade-off analysis showed that although most crosses did not outperform the native central juveniles, two of the eight hybrid crosses (SBxLS, DRxCU) demonstrated faster time to reproductive

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age and increased survival. Overall, reduced time to reach reproductive size and minimal trade-offs in at least two of the eight hybrids suggest that these crosses may accelerate and supplement recovery through natural re-seeding of genes sourced from northern reefs.

Keywords: coral, bleaching, restoration, selective breeding, hybridisation, survival, reproduction

INTRODUCTION

Local adaptation occurs when selection acts upon the standing genetic variation within populations to increase fitness under local environmental conditions (Aitken and Whitlock, 2013). It is a pervasive evolutionary process that has been documented across many kingdoms of life (Kawecki and Ebert, 2004), driven by the strength and direction of selection (Barrett and Schluter, 2008). The formation of local adaptation is generally negatively correlated with gene flow (Whiteley et al., 2015), although it is possible to maintain structured populations even under scenarios of widescale dispersal and gene flow (e.g., in marine organisms exhibiting planktonic dispersal) (Sanford and Kelly, 2011). As ocean temperatures rise, it is unclear how organisms that exhibit both high dispersal and strong signatures of local adaptation will fare. An understanding of these processes is urgent as ocean temperatures increase due to climate change and as acute thermal anomalies are becoming more frequent in the marine environment. Populations of marine organisms adapted to locally extreme thermal conditions may therefore represent reservoirs of standing genetic variation conducive to facilitating adaptation to future environmental conditions.

Reef-building corals are particularly vulnerable to ocean warming. These species are foundational to the functioning of coral reef ecosystems, but are dying at increasing rates (Hughes et al., 2018). Extensive variation in tolerance to bleaching and growth exists in corals (Jones and Berkelmans, 2011; Cunning et al., 2015a), across many habitat types (van Oppen et al., 2018), latitudes (Howells et al., 2012), and depths (van Oppen et al., 2011; Bongaerts et al., 2015). The presence of substantial standing genetic variation in key fitness traits is promising for the acceleration of thermal adaptation via the provisioning of genetic material by locally adapted populations in response to rapid rates of environmental change (Matz et al., 2017; Quigley et al., 2019). A number of novel methods for genetic management and interventions aim to facilitate the spread of adaptive genetic variation (Aitken and Whitlock, 2013). Interventions may include the movement of heat adapted adult or juvenile corals or the *ex situ* breeding of locally adapted populations (hybridisation) combined with the subsequent movement of offspring to cooler receiving reefs, generally described as Assisted Gene Flow (Aitken and Whitlock, 2013). The intent of these interventions is to increase standing genetic variation and facilitate the adaptation of populations faster than would occur under current rates of gene flow (Quigley et al., 2019).

The extent to which populations are locally adapted has important implications for the translocation of organisms and for hybridisation of organisms both within and outside their known distributions, where the extent of local adaptation can influence the magnitude of trade-offs between different traits. Trade-offs occur if a locally adapted trait results in a change of relative fitness under different environmental conditions. The correlation between local adaptation and fitness trade-offs does not appear to be strong in a survey of plant and animal taxa (Hereford, 2009) but there are limited studies examining such trade-offs in reef-building corals, especially in the wild. Several studies to date suggest a high cost of translocation. For example, fragments made from *Acropora millepora* adults reciprocally transplanted between central and southern GBR reefs had higher bleaching, increased mortality, slower growth, and changes to symbiont communities and reproductive timing on translocated reefs compared to native reefs (Howells et al., 2013). Similarly, fragments of *Porites astreoides* adult corals translocated between inshore and offshore reefs in Florida showed a high degree of local adaptation and growth trade-offs at transplanted sites (Kenkel et al., 2015).

Trade-offs involving algal symbionts and combined host-symbiont ("holobiont") responses for growth and heat tolerance are better documented. For example, trade-offs in heat tolerance and lipid composition and egg size were found in *A. millepora* hosting either *Cladocopium* or *Durusdinium* symbionts (Jones and Berkelmans, 2011). Adult *Pocillopora damicornis* corals associating with *Cladocopium* symbionts at 26°C exhibited higher growth compared to those associating with *Durusdinium,* with those differences disappearing at 29°C (Cunning et al., 2015b). However, experimental work has shown the incidence of trade-offs in adult corals to be minimal (Wright et al., 2019). Studies using juveniles produced from the hybridisation of gametes sourced from different coral populations have shown either negligible (Quigley et al., 2016) or strong effects of local adaptation (i.e., no survival benefits) (van Oppen et al., 2014), in which the difference in effect size may be driven by the outplant environment (northern × central hybrids to central site or central × southern hybrids to southern site). In the laboratory, several-fold benefits in survival and growth were recorded in hybrid juveniles with at least one warm-adapted dam and in symbiosis with *Durusdinium* symbionts (Quigley et al., 2020a). Taken together, results in corals from reciprocal translocations and population hybridisation show promise in understanding their use as intervention strategies to enhance the adaptive responses of corals to increasingly warm oceans. These differing responses suggest further work is needed to understand whether the presence and magnitude of trade-offs between traits poses a significant risk to the success of these genetic interventions.

This study looked at trade-offs associated with the "local is best" paradigm to investigate if juvenile corals with assumed higher temperature tolerance perform better or worse when transplanted to new habitats. To evaluate the benefits, risks,

and feasibility of hybridisation in the wild, we selectively bred corals from two reef regions (five reefs) across > 6° of latitude and differing thermal environments. We combined this with a common garden experimental approach to examine survival, growth, and symbiosis of purebred and hybrid corals outplanted onto the central Great Barrier Reef. We also evaluated trade-offs in their performance and modelled reproductive potential as a proxy for reef recovery potential. This approach provides both fundamental and applied knowledge regarding the adaptive potential of hybrid reef corals, lessons useful for biodiversity and ecosystem management and conservation.

MATERIALS AND METHODS

Coral Collection

Reproductively mature *Acropora tenuis* colonies were collected from three reefs in the far northern region of the Great Barrier Reef (GBR) and two reefs in the central region (**Figure 1**, ∼15 colonies per reef). These included, in the north, Curd (CU: −12.5850°S, 143.5115°E), Sand Bank 7 (SB: −13.4362°S, 143.9714°E), Long Sandy (LS: −12.5003°S, 143.7848°E), Davies (DR: −18.8217°S, 147.6495°E), and Backnumbers (BK: −18.5075°S, 147.1464°E) reefs in the central region. On average, the annual mean temperatures ranged between ∼28.7 and 29.4°C (eReefs, 01/01/2013–28/02/2018, daily measurements at 1 km resolution), with Davies representing the coolest reef (offshore, central) and Curd the warmest (inshore, north). These gravid colonies were transported to the Australian Institute of Marine Science for spawning (Townsville, Queensland, Australia). Colonies were held in outdoor holding tanks in the National Sea Simulator Facility (Seasim) at constant temperatures representative of each reef.

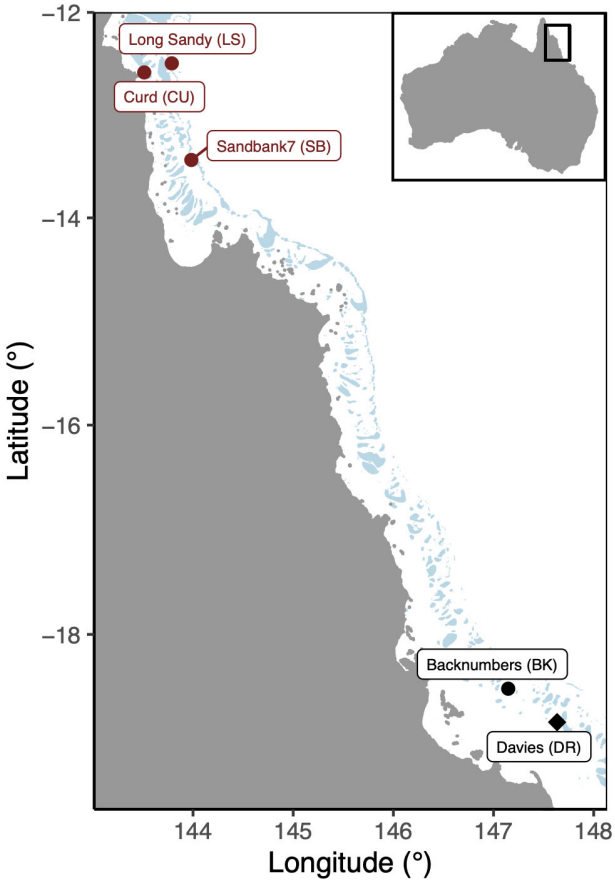

FIGURE 1 | Map of reef locations were gravid *Acropora tenuis* colonies were sourced. Northern reefs are indicated in red while central reefs are indicated in black. The transplant site ("local" cross), Davies reef, is indicated with a diamond.

Larval Rearing and Deployment

Acropora tenuis colonies were isolated at dusk from the 26th to 29th of November 2018 into individual containers. Gametes were released between 18:00 and 19:30 h and collected from the water surface. Eggs and sperm were separated through a 120 μm sieve and washed three times using 0.2 μm filtered seawater (FSW). Spawning, fertilisation and rearing followed established methods (Quigley et al., 2016). Briefly, eggs were fertilised between 21:00 and 22:00 h by adding equal numbers of eggs from one parental colony to an equal concentration of sperm from a separate parent colony standardised to 1×10^6. Sperm cells were diluted to this concentration per litre following counts using an automated sperm counter (Computer -Assisted Semen Analysis-CASA equipment). Fertilisation success was verified by visually inspecting embryos every hour for 3 h under magnification. Embryos and developing larvae from each cross were added and maintained in separate 15 L flow-through rearing cones (1 larva mL^{-1}). Additional embryos and larvae from purebred crosses were also kept in 500 L flow-through rearing tanks at the same density. In all, 17 crosses were used in this study (**Table 1**): four central dams × central sires (Central: central purebreds), four central × northern (Central-H: central hybrids), four northern × central (North-H: northern hybrids), and five northern × northern crosses (North: northern purebreds). Purebreds and hybrids distinguish intra- and inter-regional crosses, respectively. All potential cross combinations successfully produced larvae, but only 17 were selected for field deployment due to permit constraints to the number of tiles outplanted at the selected field site.

Larvae were settled in two batches, from the 5th to the 10th of December and again from the 11th to the 16th of December in 2018. Larvae from each cross were added to separate flow-through tanks per cross (1 μm FSW) containing terracotta tiles (n = 1,474 tiles) with added crushed crustose-coralline algae to induce settlement. These 1,474 tiles were laid flat across the bottom of each of the tanks or alternatively, hung vertically in groups of 10 tiles (separated by spacers). After the first batch of tiles was removed, a second set of tiles (plus freshly crushed crustose algae) were added to those tanks. Tiles were then transferred to flow-through outdoor tanks set at 27.5°C (1 μm FSW) that contained adult *A. tenuis* from Davies reef as a source of Symbiodiniaceae inoculation and held until deployment.

In March 2019, a total of 1,171 tiles with 3,748 juveniles were deployed to Davies reef using SCUBA (**Figure 1** and

TABLE 1 | Summary of sample sizes of juvenile corals analysed for each of the 17 reproductive crosses.

Cross type	Dam	Sire	Crosses used	Survival		Growth	Colour
			(DamxSire)	Day 0 (n)	Day 217 (n)	(n)	(n)
Central purebred	Central	Central	BKxBK	99	17	7	17
			BKxDR	14	3	2	3
			DRxBK	30	6	5	6
			DRxDR	74	16	6	15
Central hybrid	Central	Northern	BKxCU	87	17	10	17
			BKxLS	62	8	3	6
			DRxCU	26	6	4	5
			DRxSB	48	8	4	8
Northern hybrid	Northern	Central	CUxBK	83	11	5	9
			CUxDR	124	12	6	10
			LSxBK	49	6	4	5
			SBxDR	41	5	0	4
Northern purebred	Northern	Northern	CUxCU	107	14	7	13
			LSxLS	153	11	8	11
			LSxSB	86	3	2	2
			SBxLS	6	3	2	3
			SBxSB	113	16	10	16

Purebreds include central × central or northern × northern crosses whilst hybrids refer to central × northern or northern × central crosses. Sample sizes of juveniles (n) are listed for survival, growth, and colour analyses. Source reefs are abbreviated as in ***Figure 1*** *and are as follows: Backnumbers (BK), Davies reef (DR), Curd reef (CU), Long Sandy reef (LS), Sandbank 7 (SB).*

Supplementary Table 1). Tiles were placed onto rods; rods were fixed into cassettes (two rods each); and cassettes were attached onto seven frames that were secured onto the sand at Davies reef. Each frame (1.074 × 2.9 m) contained 16 cassettes that held two rods of 10–16 tiles each. Tiles were orientated vertically and were evenly spaced with 1.5 cm PVC spacers to allow for growth. The position of each tile, rod, cassette, and frame was recorded during tile deployment.

Survival, Growth, and Colour

Survival, growth, and coral colour were measured immediately prior to deployment and after 91 and 217 days in the field. On days 91 and 217, tiles were retrieved using SCUBA, gently cleaned with a silicone brush, and juveniles were assessed by eye and then photographed, then the tiles were re-deployed into the exact same position. A final number of 1,202 juveniles were recorded as alive (including individuals suffering tissue loss) or dead.

Growth and colour were measured from digital images. Three solitary juveniles were selected per photograph for growth and colour measurements. Prior to deployment, juveniles were photographed using a Nikon D810 camera body, Nikon AF-S 60 mm micro lens, with each photo including a 2 cm scale bar and CoralWatch Health Chart (image resolution 7,360*4,912 pixels; Siebeck et al., 2006). In the field, each juvenile was assessed by eye and photographed in the field with an Olympus Tough TG-5 camera with a 15 cm scale bar and CoralWatch Health chart (image resolution 4,000*3,000 pixels) and Ikelite DS160 strobes attached to a stationary set-up. The camera was fixed within an Olympus PT-058 waterproof housing, X-Adventurer M1000 video light, and Olympus UFL-3 flash in a fixed frame for constant angle and distance. The housing was mounted onto a Hyperion pro tray and attached to a custom high-density polyethylene frame for consistency in angle and distance per tile photograph. For all photos, the camera lens was positioned 190 mm from the tile, consistent flash settings were used, and the frame contained a built-in coral health chart and scale bar.

Juvenile area was used to calculate growth, it was measured from the images using the polygon selection tool and calibrated to a 10 mm scale in ImageJ2 software (Rueden et al., 2017). The colour of the juveniles was used to indicate changes in Symbiodiniaceae cell density and/or chlorophyll content. Juvenile colour was matched to the closest category in the CoralWatch "D" coral health chart in each image by a single person to minimise observer bias.

Statistical Analysis

Survival was analysed using Kaplan-Meier survival curves in the R packages "survival" and "survminer" (Therneau, 2015; Kassambara et al., 2017). To evaluate differences in survival between the purebred and hybrid crosses, survival curves were estimated for each type with juveniles grouped by each cross. Survival curves also were estimated for each cross individually and compared to the local purebred juvenile corals at the outplant locations (DRxDR).

Pairwise *post hoc* tests were used to compare data collected from the purebred and hybrid crosses using the Peto and Peto test from the "survminer" package (Kassambara et al., 2017) and *p*-values were corrected for multiple comparisons using the Benjamini-Hochberg method of controlling the false discovery rate (Benjamini and Hochberg, 1995).

Growth

Linear growth rates were estimated for 85 juveniles that had two or more area measurements. Changes in mm^2 area per day were analysed using a linear mixed model with type of cross as a fixed effect and settlement tile as a nested random effect to account for tiles with multiple juveniles (Bates et al., 2014; Kuznetsova et al., 2017). Residuals were inspected for normality and heterogeneity of variance.

To estimate the time until the juveniles from different crosses would reach reproductive size, exponential growth curves [$A_t = A_0{}^*(1 + r)^t$] were fitted using the R package "nlme" (Pinheiro et al., 2014) to the average areas (A) at day 0, 91, and 217 for each cross. Previous field-based measurements for this species suggest that reproductive maturity (∼4–5 years) is reached at a diameter of 20–25 cm (Iwao et al., 2010). This range of sizes is equivalent to a circular surface area of approximately 314 cm^2 when converted using πr^2. We solved the exponential growth curve for t when the area at time t (A_t) was 314 cm^2. The median time required to reach this size was compared for each cross type. For comparison, the same calculation was applied to *A. tenuis* transplanted to Magnetic Island sourced from a similar field-based experiment using purebred juveniles from that reef (Quigley et al., 2020b), which is situated almost directly inshore of Davies reef (**Figure 1**).

Colour

The proportion of juveniles in each CoralWatch Health Chart category were analysed using a multinomial model with cross type and time as predictors and equidistant categories. A second multinomial model was used to determine whether changes in colour score corresponded to individual crosses rather than types of crosses.

RESULTS

For survival and colour analysis, juveniles growing in contact with other juveniles or those that were dying (i.e., losing tissue) were not considered (i.e., filtered from the data), leaving 1,201 juveniles on 546 tiles for survival analysis and 768 juveniles and 509 tiles for colour analysis. Growth rates were measured per juvenile and included 85 juveniles with repeated area measurements across al timepoints.

Survival

Survivorship decreased from 100% at deployment (n = 1,202 filtered juveniles) to 30.8% ± 4.2 SE after 91 days and then to 17.1% ± 2.5 after 217 days in the field (**Figure 2A**). Survivorship curves differed significantly amongst the four types of crosses (central purebreds, central hybrids, northern purebreds, northern hybrids; [$\chi^2(16) = 56.5$, $p < 0.001$]. Central crosses had significantly higher survival compared to northern purebred and northern hybrid crosses (both $p \leq 0.001$). Similarly, central hybrid crosses had significantly higher survival compared to northern purebreds ($p = 0.050$) but not northern hybrid crosses ($p = 0.112$). There was no significant difference in survival between central purebreds and central hybrids ($p = 0.112$) or between northern purebreds and northern hybrids ($p = 0.695$).

In addition to variation amongst the types of crosses, individual crosses exhibited significant differences in survivorship [$\chi^2(16) = 48.1$, $p < 0.001$], predominately driven by the high variation in northern purebreds crosses (**Figure 2B** and **Supplementary Table 2**). The northern purebred cross, SBxLS, had the highest survival. However, due to the low initial number of SBxLS juveniles at time of outplant (**Table 1**), standard error was high, contributing to the lack of significant differences between this cross and the central purebred cross DRxDR ($p = 0.158$). SBxLS did have significantly higher survival compared to other northern hybrids, northern purebreds, and three of the four central hybrid crosses (all $p < 0.046$). In contrast, LSxSB had significantly lower survival compared to all other crosses (all $p < 0.041$). The local cross, DRxDR, had significantly higher survival compared to the two northern purebred and one northern hybrid crosses (CUxDR, LSxLS, LSxSB; all $p < 0.008$). These three northern crosses had the lowest survival and both LSxLS and CUxDR had significantly lower survival compared to three central purebred crosses, including DRxDR (all $p < 0.045$). In summary, survival was highly variable by cross and the direction of each cross, but overall, the local cross did perform better than many of the hybrid crosses, with the exception of SBxLS and CUxDR.

Growth and Time to Reproduction

Juvenile *A. tenuis* doubled in size after 217 days in the field, growing from 1.3 ± 0.1 SE mm^2 ($n = 65$) to 2.6 ± 0.1 mm^2 ($n = 51$, **Figure 3A**). SBxLS (3.8 ± 0.1 mm^2) and DRxSB (3.6 ± 0.4 mm^2) were the largest juveniles after 217 days in the field. Growth rates were measured per juvenile ($n = 85$), which prevented estimation of the growth rate of SBxDR juveniles, where no SBxDR juveniles were measured more than once. The average growth rate of all crosses was 0.007 ± 0.010 mm^2 day^{-1} (0.255 cm^2/year). Growth rates were similar amongst the four types of crosses [**Figure 3B**; $F_{(3,\ 72.1)} = 1.7$, $p = 0.17$] and amongst individual crosses [$F_{(15,\ 54.5)} = 0.9$, $p = 0.558$].

For comparison, exponential growth rates of *A. tenuis* in this study were compared to previous growth rates measured for *A. tenuis* juveniles (**Figure 4A**). Growth rates of *A. tenuis* at Davies reef in this study (−0.1 to 0.6% area per day) were much slower than previous values for this species (1.8% area per day; **Figure 4B**). Exponential growth rates in this study ranged from −0.07 (BKxBK) to 0.57% area per day (LSxBK) and followed an approximately normal distribution (**Figure 4B**). By extrapolating these growth rates, we calculated the time until the juveniles would reach reproductive size. Central purebred crosses would take approximately 12.5 years (median) to reach reproductive size, northern purebreds would take only 7.9 years, and the hybrids (northern and central) would take 9.3 years to reach reproductive size (**Figure 4C**).

Colour

The distribution of juvenile colour scores (D1–D6; $n = 506$) measured with the Coral Colour Reference Card changed significantly over time [**Figure 5A**; $\chi^2(10) = 61.8$, $p < 0.001$ but

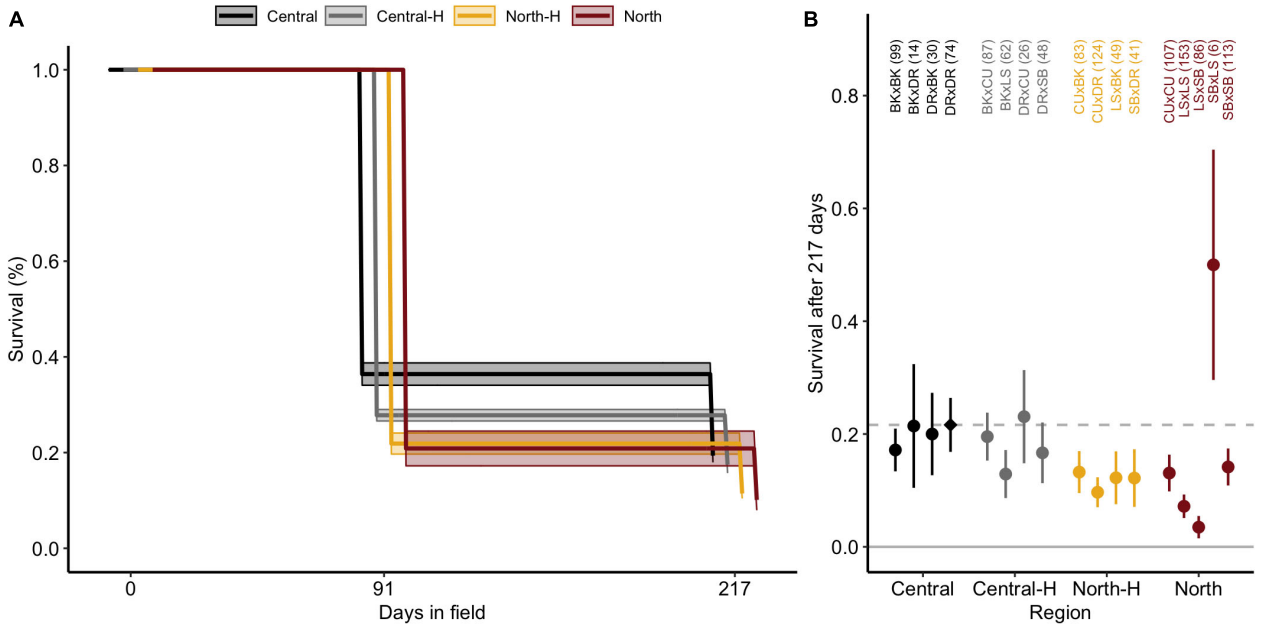

FIGURE 2 | Survival of *Acropora tenuis* juveniles over 217 days at Davies reef. **(A)** Kaplan-Meier survival curves for juveniles from each type of cross. Central and North represent the regional purebred crosses while Central-H and North-H represent the regional hybrid crosses (according to dam). Lines and shaded regions indicate estimated survival and ± confidence intervals (CI), respectively, and are staggered to aid interpretation. **(B)** Mean survival of *A. tenuis* crosses grouped and coloured by cross. Error bars indicate ± standard errors (SE). The dashed line and diamond symbol delineate the mean survival for the local cross (DRxDR) to aid in comparison. Crosses are abbreviated as in **Figure 1**, and day 0 sample sizes are listed in parentheses.

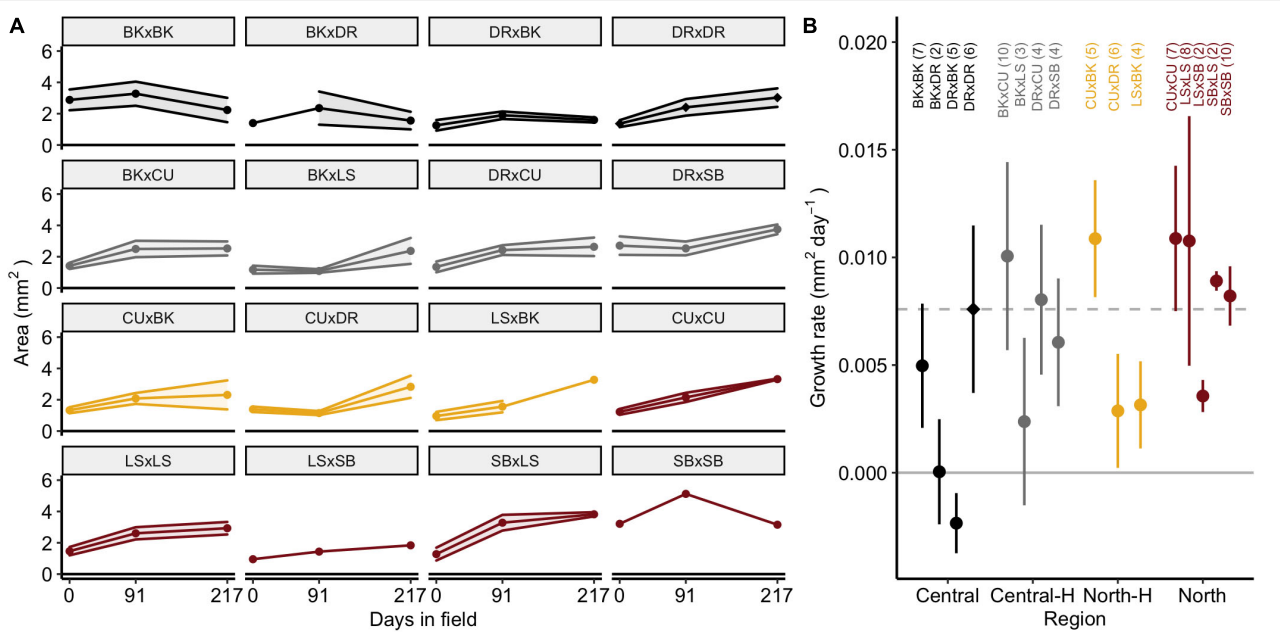

FIGURE 3 | Size and growth rate of *Acropora tenuis* juveniles. **(A)** Area (mm^2) of juveniles over 217 days in the field. Panels depict crosses in alphabetical order and are coloured according to the type of cross. Points represent means and shaded regions represent ± SE. **(B)** Growth rates of juveniles (mm^2 day^{-1}). Central and North represent the regional purebred crosses while Central-H and North-H represent the regional hybrid crosses (according to dam). Points represent means and error bars represent ± SE. The dashed line and diamond symbol indicate the local cross (DRxDR). Crosses are abbreviated and shaded according to cross as described in **Figure 1**. Sample sizes are listed in parentheses.

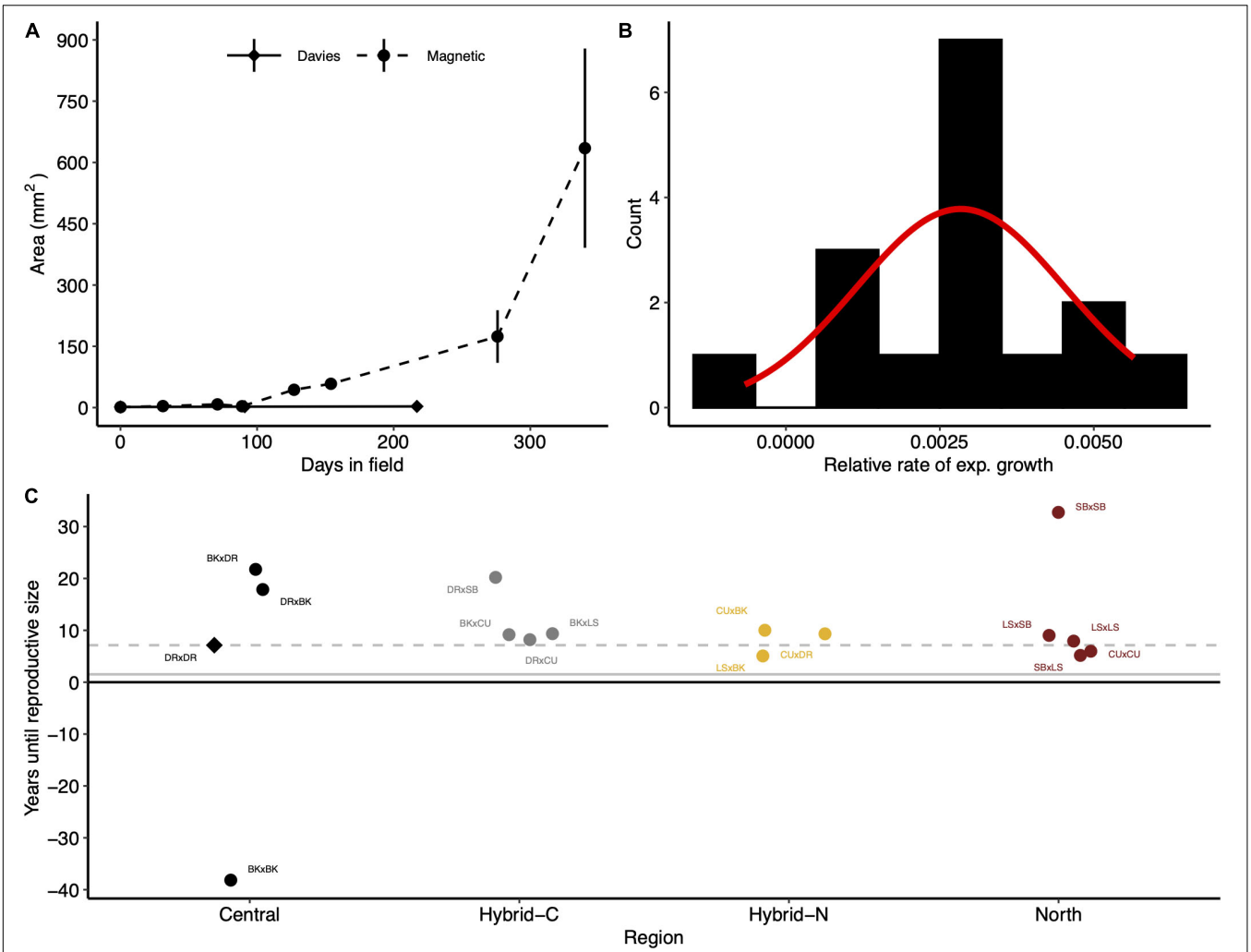

FIGURE 4 | Exponential growth and estimated time until reproductive size for *Acropora tenuis* juveniles. **(A)** The area (mm^2) of juveniles over time deployed at Davies reef (circles; this study) and Magnetic Island (triangles; Quigley et al., 2020b). Points and error bars represent means and ± SE, respectively. **(B)** The distribution of the relative growth rate estimates for crosses transplanted to Davies reef. The red line indicates expected counts based on a normal distribution with a mean and SD equal to the relative growth rate estimates. **(C)** The predicted time (years) until transplanted juveniles reach reproductive size (20 cm diameter). Points represent means of individual crosses grouped and coloured by the type of cross. Central and North represent the regional purebred crosses while Central-H and North-H represent the regional hybrid crosses (according to dam). The grey dashed line indicates the number of years until the local cross (DRxDR, diamond) reaches expected reproductive size. Crosses are abbreviated as in **Figure 1**.

was overall similar amongst some types of crosses [$\chi^2(15) = 15.2$, $p = 0.436$] and amongst cross types over time [$\chi^2(30) = 30.8$, $p = 0.428$]. Although the distribution of colour scores changed over time (with some colour scores decreasing in time), the median score was relatively stable between 5–6 (**Figure 5A**). At deployment, most juveniles were in the highest pigmentation categories of 5 and 6. By days 91 and 217 of field deployment, colour scores had decreased, indicating a paling of juveniles. Specifically, the largest change in colouration occurred between outplant and day 91, with little change in colour occurring between days 91 and 217 (**Figure 5A**). For individual colour score categories, the proportion of 5 categories decreased significantly by day 91 (33%; $p < 0.001$) while the proportions of 1 and 2 scores increased significantly by day 91 (1: 7%, $p < 0.026$; 2: 23%, $p < 0.001$). Across each of the colour categories, none of the crosses changed significantly between days 91 and 217 (all comparisons < 7.6%; all $p < 0.163$). On average, colour scores were 5.47 ± 0.04 at deployment, which decreased to 4.45 ± 0.23 by 91 days, and then rose to 4.69 ± 0.17 by 217 days.

Colour scores amongst individual crosses changed over time [$\chi^2(5) = 135.5$, $p < 0.001$], were similar amongst individual crosses [$\chi^2(80) = 81.0$, $p = 0.447$], and amongst individual crosses over time [$\chi^2(80) = 32.0$, $p = 1.000$]. Juveniles from the local cross DRxDR, also decreased in colour over time (black diamonds, **Figure 5B**). After 217 days in the field, 7 of the 17 crosses maintained colour scores > 5, four of which were northern hybrids (CUxBK, CUxDR, LSxBK, SBxDR), two central hybrids (DRxCU, DRxSB) and one northern purebred cross (LSxLS).

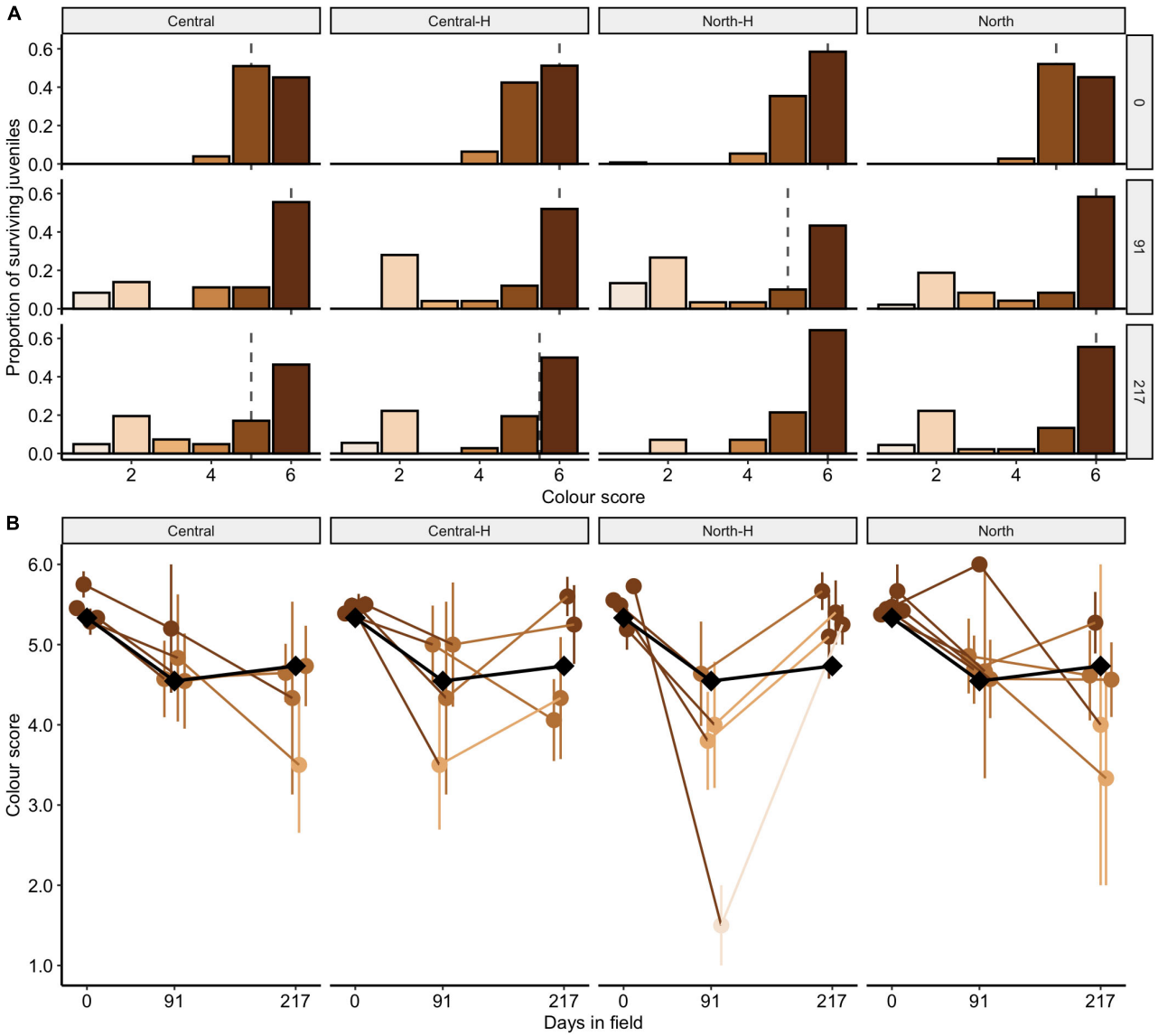

FIGURE 5 | Colour scores for transplanted *Acropora tenuis* juveniles. **(A)** The distribution of colour scores (1–6) for each cross after 0, 91, and 217 days in the field. The panels distinguish between cross types and days deployed. Central and North indicate regional purebreds while Central-H and North-H indicate regional hybrids (according to dam). The height of the bars represents the proportion of juveniles in each colour category and the dashed line indicates the median colour score. Bars are coloured to match the CoralWatch "D" colour score. **(B)** Mean colour scores of crosses over time. Cross are labelled as in **(A)**. Points and error bars represent the mean and ± SE, respectively. The local cross, DRxDR, is indicated by black diamonds and lines. The colour of points and lines for other crosses match the CoralWatch "D" colour score.

DISCUSSION

The use of intervention methods in coral reef conservation is accelerating (National Academies of Sciences Engineering and Medicine, 2019). To assess the potential risks and benefits associated with these methods, field testing is essential (van Oppen et al., 2014; Quigley et al., 2016). This study provides key baseline field data for assessing the feasibility of hybridisation and answers outstanding questions relating to trade-offs between growth and survival and reproductive potentials of corals produced from AGF methods.

Trade-Offs

Trade-offs are often defined as the increase in the mean value of one trait at the expense of decreases in another, but trade-offs may instead be associated with locally adapted traits and populations (Hereford, 2009), in which moving organisms to new habitats disrupt traits when placed in new environments. Relative to the baseline cross from the deployment reef (DRxDR), there was little evidence of classic patterns in trade-offs in growth and survival from hybrid crosses (Central-H or North-H), with both of these groups (including the individual crosses within them), exhibiting characteristics of both low survival/high growth or low

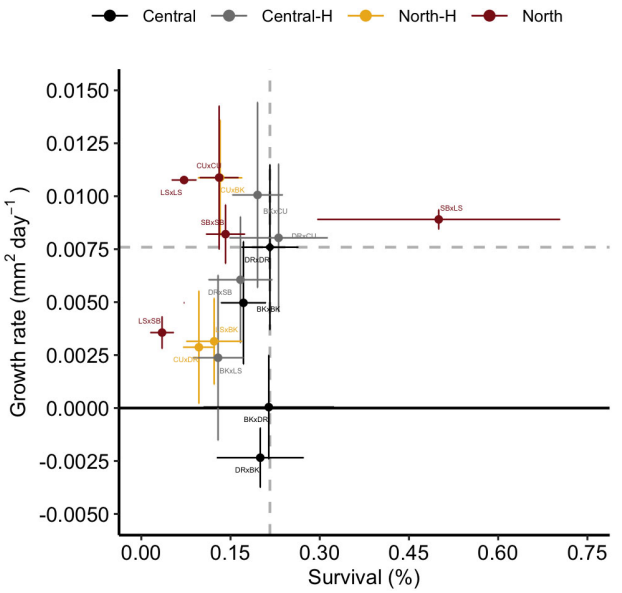

FIGURE 6 | Growth rate vs. survivorship of *Acropora tenuis* crosses. Points represent the mean growth rate and survival of juveniles in each cross. Points represent means and error bars represent ± SE and are coloured according to the type of cross. Central and North indicate regional purebreds while Central-H and North-H indicate regional hybrids (according to dam). Dashed grey lines and a black diamond indicate the mean growth rate and survival of the local cross, DRxDR. Names of crosses are abbreviated according to **Figure 1**.

growth/low survival (**Figure 6**). Specifically, three of the eight hybrid crosses (BKxCU ∼/ = DRxCU < SBxLS) did not exhibit any trade-off between growth and survival, exhibiting both high growth and survival relative to local DRxDR juveniles, thereby suggesting potentially higher overall fitness for these crosses. A minority (one of eight) of the other hybrid crosses exhibited lower survival but higher growth (CUxBK), with the remaining hybrids (five of eight of the crosses) exhibiting lower growth and survival relative to central purebreds (DRxSB, LSxBk, CUxDR, BKxLS, and LSxSB). Interestingly, neither BKxDR or DRxBK juveniles did well at Davies reef, exhibiting both low growth and low survival.

Trade-offs have been examined in corals for traits like survival, growth, and reproduction (Jones and Berkelmans, 2011; Cunning et al., 2015a; Quigley et al., 2020a). Three of the hybrid crosses repeated above did not exhibit any trade-off between these traits. Furthermore, both central hybrids and northern hybrids exhibited characteristics of both low survival/high growth or low growth/low survival, suggesting there was no consistent trade-off between crosses by GBR region but may instead be reef specific. These results mirror multi-factorial tank experiments, where specific genotypes were the main factor influencing phenotypic variation (Wright et al., 2019). Indeed, the assumption formed across terrestrial and aquatic ecosystems is that trade-offs are a persistent feature in ecosystem ecology (Sgrò et al., 2011), although the pervasiveness of trade-offs are likely overstated and, if present, they are likely weak (Hereford, 2009).

The mean survival rate of juveniles after 217 days in the field was high relative to estimates from other reef locations. Post-settlement survival in the field is often low for highly fecund species, including corals (reviewed in Randall et al., 2020) and generally estimated to be between 0.6 and 2% for juveniles within the first 12 months of life (Doropoulos et al., 2019). It is important to note that survival from the literature are generally for purebred corals outplanted to their native reefs. However, survival between population crosses was variable, and when we examined purebred survival rates for juveniles outplanted to their natal reef (DRxDR juveniles), rates also were high (∼20%) and provide an important ground-truthing for baseline survival rates. Decreased survival rates from the literature relative to ours may be attributed to higher densities of juveniles on substrates, in which overcrowding may lead to high mortality (Cameron and Harrison, 2020). A vast majority of juveniles in this study were found on single tiles with adequate space for growth and low competition and all were singles (no clumps). Finally, age-at-outplant is also important for influencing survival, with the lowest survival generally occurring within the first few months of life (reviewed in Doropoulos et al., 2019). Our high survival rates may therefore have been facilitated by the more advanced age of juveniles, and therefore increased size, at the time of outplanting to the field (∼4 months).

One of the key concerns with the adoption of hybridisation is the negative impact on growth rates of individuals produced and transplanted to non-native sites due to the influence of local adaptation. Growth has been identified as one of the potential key performance indicators for restoration success, with normalised summer growth rates the best predictor of rapid growth later (Edmunds and Putnam, 2020). Sizes and growth rates are important as both are linked to reproductive timing. For example, if growth decreases by 2% in non-native crosses, reaching reproductive size may be delayed, leading to a decreased efficacy for the spread of propagules from this intervention. This lag in growth rate is particularly relevant when non-natives are transplanted to colder environments (northern to central GBR), in which rates are expected to decrease (van Oppen et al., 2014) compared to the increased rates when outplanted to warmer climes (Browne et al., 2019). Growth rates in purebred northern crosses were greatest overall, followed by the hybrid crosses and then purebred central crosses, suggesting no decreases in growth rate. These higher growth rates for northern purebred corals in cooler climates instead suggest that risks associated with lineage swamping are more likely (Aitken and Whitlock, 2013). Although we only sourced reefs from two regions here (northern and central GBR), these results suggest that the use of lab-produced hybrids may ameliorate some of this risk given their mixed genomic backgrounds.

Comparisons to known growth rates in the field suggest juvenile crosses at Davies grew slowly. Extrapolating our 0.007 ± 0.010 SE mm^2 day^{-1} estimates results in growth of 0.255 cm^2/year, which is well under growth rates for outplanted juveniles that have been estimated at 1 cm/year (Doropoulos et al., 2015) to 4–5.2 cm per year (Iwao et al., 2010; dela Cruz and Harrison, 2017). Slow growth at Davies is further corroborated from *A. tenuis* juveniles collected from Magnetic Island (Quigley et al., 2020b). When converted to reproductive size (diameter

of ∼20 cm), the Davies juveniles would take 9.1 years to reach that diameter. Faster inshore growth has been reported for adult-derived fragments (Rocker et al., 2019), and it is likely tied to increased nutrients loads on inshore reefs. Hence, the slower growth found here is likely influenced by settlement substrate, light environment, and nutrient conditions of the native reef.

At time of outplant in March, most juveniles were pigmented owing to the symbionts provided from Davies reef conspecific adults. Whilst bleaching can be defined as paling of coral tissues either through the decreased density of Symbiodiniaceae or photobleaching of symbiont pigments (Hoegh-Guldberg, 1999), the paling observed here may be attributed to a recalibration of standing-stock densities. The recalibration could be driven by the change in light conditions from within the rearing facility at the Seasim (∼50–100 photosynthetically active radiation–par, μmol photons m^{-2} s^{-1}) compared to much higher values recorded in the field, which can range from ∼130 to 550 par (Abrego et al., 2009; Jones et al., 2016; Ricardo et al., 2017). Ocean temperatures during this period of assessment were not warmer than average, and the assessments were finalised before the 2020 bleaching event, therefore paling was unlikely due to higher temperatures. Symbiont communities are highly dynamic in coral early life stages (both in regards to infection densities and taxonomic composition, Quigley et al., 2017a), and thus the decrease in densities may not indicate a physiological breakdown between coral and symbiont, but instead a calibration to steady-state dynamics for the reef light environment or seasonal variation in light (Fitt et al., 2000). Interestingly, this same pattern in the reduction of symbiont densities was observed in Magnetic Island juveniles of the species *Acropora tenuis* after 167 days in the field (Quigley et al., 2020a) and may be a persistent characteristic of coral ontogeny linked with the processes driving the winnowing of symbiont communities.

The risk of incompatibility between host genotype and the symbiont community available for inoculation at Davies reef was also a concern given that the availability of free-living symbiont communities varies across the GBR (Quigley et al., 2017a) and the host genetic identity plays a role in determining the endosymbiont community (Quigley et al., 2017b). However, we did not observe a significant decrease in colour (as a proxy for symbiont densities, see Siebeck et al., 2006) in crosses produced with one or both parents from northern colonies (as reflected in lack of statistical significance between regions over time or between regions overall), which suggests that the paling observed here was not due to an incompatibility between host genetics from northern reefs and symbionts available from central reefs.

Reproduction and Recovery

There is concern that the application of genetic conservation practices may result in offspring that will grow slower (diminishing intervention efficacy) or faster (swamping locals) compared to native offspring (Aitken and Whitlock, 2013). Although a trait like acceleration of growth rates may be desired for restoration goals, information on the limits of growth is essential. To predict how long it would take for offspring produced via AGF to reach reproductive size, estimates of size to reproduction were calculated. Time to reproduction was shortest for northern purebres and longest for central purebreds. Given that the production of coral propagules is costly in both time and resources at land-based facilities, enhancing natural reproductive cycles in the wild is recommended. Hence, the enhancement of the spread of alleles associated with heat tolerance will benefit from AGF stock becoming incorporated into the natural reproductive cycles of reefs, diminishing the reliance on the artificial production of coral offspring and re-seeding (Quigley et al., 2019). Although our results only represent reefs from two regions on the GBR, given this, northern purebred crosses would kickstart self-seeding intervention efforts faster compared to other crosses, reducing the need for future *ex situ* reproduction and field deployment. The potential for increased recovery due to growth and reproduction of northern purebreds in central reefs may be balanced by limiting the total number outplanted to balance for "migrational meltdown" due to maladaptive alleles (Matz et al., 2018; Baums et al., 2019). Enhancing the spread of alleles may also rely on the fertility of AGF produced corals as it is a key indicator of recovery potential (Álvarez-Noriega et al., 2016), and should be a future area of research.

Recovery of reefs in terms of coral cover would therefore be expected to be faster with the use of northern purebred corals as they would be able to produce sexually-derived propagules the sooner. Recovery potentials via reproductive rates are intrinsically tied to growth rates in corals, with colony size as a well-known predictor of fecundity. Interestingly, initial area did not seem to affect the time until reproduction. As colony size increases in corymbose morphologies like *A. tenuis*, polyp maturity tends to increase exponentially and oocyte number and reproductive investment tend to increase linearly (Álvarez-Noriega et al., 2016). Furthermore, recovery of coral cover is aided by larger corals that occupy more space (i.e., fewer individual colonies for the same recovered size per unit area of reef), where corals of ∼40 cm in diameter have been found to be of particular value for ecosystem recovery (Ortiz et al., 2009). This size therefore provides a good target for the selective breeding of corals with coral cover recovery as an objective.

Interestingly, SBxLS was the population cross that performed the best in terms of highest survival, fastest growth, and the least amount of paling. Although the number of individuals from this cross was low relative to other crosses in this experiment (**Table 1**), individuals exhibited both high survival and high growth. Indeed, this signature is suggestive of density-independent mortality, in which a few crosses consisting of a smaller number of individuals exhibited high relative survival whereas other crosses composed of a greater number of juveniles experienced lower relative survival. This suggests that genotype specific differences exist between colonies and those are provisioned to offspring. This response has been observed before in outplants of northern crosses at central reefs (Quigley et al., 2016), although a similar result was not observed when juveniles were transplanted to southern reefs (van Oppen et al., 2014). Finally, although the overall sample size was low for the final number of surviving SBxLS juveniles, the pattern in

high survivorship was mirrored in experimental systems in which juveniles of this cross exhibited close to 100% survival at 27°C and 75–100% survival at 32°C across multiple symbiont treatments after 58 days (Quigley et al., in review). Hence, this cross appears to be particularly hardy across a range of temperatures and conditions.

CONCLUSION

Coral reefs are facing accelerated rates of environmental change, especially continued ocean warming and more severe marine heat waves. Here we show that coral reefs and other environments shaped by disturbances and pressures that have led to potentially strong signatures of local adaption may benefit from interventions focussed on incorporating selective breeding to produce offspring of hybrid stock sourced from warmer locations in the northern GBR, which may match projected future conditions. Although we show that trade-offs in key traits during non-warming years were minimal in a few of the hybrid crosses (25%), the next steps include exposing these crosses to warming to assess their performance under stress. The lack of a consistent pattern in trade-offs between growth and survival of juveniles in these field trials also suggests that perhaps practitioners should look beyond the "local is best" paradigm. This field-based experiment provides an experimental demonstration in the wild for this type of method, thereby contributing essential information that is needed to assess some of the key risks associated with genetic interventions and it informs decision making about the utility of hybridisation for reef restoration. Finally, whilst interventions aimed at accelerating the natural rates of adaptation to heat tolerance to preserve some level of coral reef functionality and diversity are valuable, these interventions must be considered in conjunction with strong action on climate change.

DATA AVAILABILITY STATEMENT

The data and scripts will be made available on Github (https://github.com/LaserKate/AGF2018Field).

AUTHOR CONTRIBUTIONS

KQ, LB, and MvO designed the research. KQ, MM, DA, LB, and GM performed the fieldwork. BR and KQ analysed the data. KQ, BR, MM, LB, and DA wrote the manuscript. All authors contributed to the critical review of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.636177/full#supplementary-material

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frontiers in Marine Science

POLICY AND PRACTICE REVIEWS
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Perspectives on the Use of Coral Reef Restoration as a Strategy to Support and Improve Reef Ecosystem Services

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In 2019, the United Nations Environment Assembly requested that the United Nations Environment Programme (UNEP) and the International Coral Reef Initiative (ICRI) define best practices for coral restoration. Guidelines led by the UNEP were prepared by a team of 20 experts in coral reef management, science, and policy to catalog the best-available knowledge in the field and provide realistic recommendations for the use of restoration as a reef management strategy. Here, we provide a synthesis of these guidelines. Specifically, we present (1) a case for the value of coral reef restoration in the face of increasing frequency and intensity of disturbances associated with climate change, (2) a set of recommendations for improving the use of coral reef restoration as a reef management strategy, tailored to goals and current methods. Coral reef restoration can be a useful tool to support resilience, especially at local scales where coral recruitment is limited, and disturbances can be mitigated. While there is limited evidence of long-term, ecologically relevant success of coral reef restoration efforts, ongoing investments in research and development are likely to improve the scale, and cost-efficiency of current methods. We conclude that coral reef restoration should not be seen as a "silver bullet" to address ecological decline and should be applied appropriately, with due diligence, and in concert with other broad reef resilience management strategies.

Keywords: coral restoration, climate change, recommendations, intervention, efficiency, scalability

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INTRODUCTION

With dramatic declines in coral cover worldwide, especially in the last 3–5 years (Pandolfi et al., 2003; Hughes et al., 2017, 2018), it has become clear that bolder actions are necessary at both global and local scale to secure a future for coral reefs. Coral reef restoration, in particular, is increasingly employed as a management strategy to halt declines in coral cover and support reef resilience. Increased interest in coral reef restoration is illustrated by the central role restoration is taking in national and international commitments under various multilateral environmental agreements. For example, the United Nations General Assembly has put "rehabilitating our environment" at the heart of the 2030 Agenda for Sustainable Development and declared 2021–2030 as the UN Decade on Ecosystem Restoration. The 4th United Nations Environment Assembly in 2019 also passed a

resolution specific to the sustainable management of coral reefs (Resolution 4/13) recognizing the role of restoration to achieve biodiversity goals (United Nations Environment Assembly (UNEA), 2019). A recent ICRI report (McLeod I. M. et al., 2019) revealed that 88% of ICRI members are interested in the development of new international commitments and policies specifically dedicated to coral reef restoration. At the national level, initiatives such as the Reef Restoration and Adaptation Program in Australia (RRAP, Bay et al., 2019), NOAA's restoration strategy within the coral reef conservation strategy (National Oceanic and Atmospheric Administration (NOAA), 2018), the Coral Reef Restoration Protocol in Costa Rica (AIDA-Americas, 2019), or specific Coral Reef Action Plans in Thailand (Suraswadi and Yeemin, 2013) highlight increased interest in investing in coral reef restoration.

However, some confusion arises from an active debate among coral reef scientists on the value of coral reef restoration in the face of large-scale disturbances such as warming temperatures and increased ocean acidification. Two IPCC reports (IPCC, 2018; Bindoff et al., 2019) summarize the existing projections of future coral bleaching to state that coral reefs as we know them will all but disappear in a scenario of up to 2°C warming, and up to 90% of coral reefs could be lost even with an increase of 1.5°C. In this context, many experts argue that coral reef restoration is merely a band-aid solution and a distraction from global actions on threat reduction (Bellwood et al., 2019; Morrison et al., 2020). Other experts argue that even if greenhouse gas emissions were to be drastically reduced immediately, global ocean temperatures could still take decades to stabilize (Hansen et al., 2007), and that bold active management actions at the local level such as coral reef restoration are necessary to sustain and re-build reef ecosystems, alongside climate action and protection measures (Rinkevich, 2019; Duarte et al., 2020). Climate action, albeit critical, is only one part of the big equation we need to solve to ensure a future for coral reefs, and restoration can create a necessary bridge to rescue corals at local scales while global threats are being addressed (Coral Restoration Consortium (CRC), 2020).

Adding to the confusion is the largely experimental nature of the practice coral reef restoration (Bayraktarov et al., 2016, 2020; Hein et al., 2017; Boström-Einarsson et al., 2020). Apart from a few notable examples of positive long-term outcomes (In Fiji Coral for Conservation, 2020; in Belize Fragments of Hope, 2020), there is limited evidence that it can be an effective management strategy to support reef resilience. A lack of long-term monitoring of existing projects (coral restoration projects have a median monitoring duration of 12 months, Boström-Einarsson et al., 2020), and reporting of success focused on a few technical metrics (e.g., coral growth and survival) rather than metrics related to ecosystem function and health or socio-cultural and economic outcomes (Hein et al., 2017; Boström-Einarsson et al., 2020) make it difficult to assess and share general best practices (Leocadie et al., 2020). In the last few years, there has been an explosion of research and development on cutting-edge solutions to scale-up current coral reef restoration techniques (National Academies of Sciences Engineering and Medicine (NASEM), 2019; Bay et al., 2019, RRAP). These developments are necessary to help corals persist. However, the novelty of this research creates a gulf between existing practices and what is recommended, leaving managers, practitioners, decision-makers, and funding agencies with a lack of guidance for what coral restoration can realistically achieve.

In 2019, the United Nations Environment Assembly adopted Resolution 4/13 on sustainable coral reefs management requesting UNEP and ICRI to better define best practices for coral restoration, as appropriate, for the maintenance of ecosystem services, including for coastal defense and restoration of fish nursery areas. In response, a report was prepared by 20 global coral reef restoration experts to assist practitioners, managers, and decision-makers in deciding whether and how to use of coral reef restoration as a strategy to protect coral reefs locally, regionally, and globally (Hein et al., 2020a, UNEP). Here, we synthesize these experts' perspectives on: (a) goals and methods of coral reef restoration on the eve of the UN Decade on Ecosystem Restoration; (b) arguments for and against restoring coral reefs in the face of climate change; and (c) recommendations on how current methods can be used for particular goals and situations.

CORAL REEF RESTORATION ON THE EVE OF THE UN DECADE

Ecological restoration is defined by the Society for Ecological Restoration (SER) as *"the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed"* (Society for Ecological Restoration International Science and Policy Working Group, 2004). In the past, the goal of restoration has been to restore an ecosystem back to a historical baseline. This view also implied that the threat(s) responsible for the degradation, damage or destruction could be removed. However, this may not be possible for coral reefs because the threat of rising ocean temperatures and ocean acidification will continue for decades even if greenhouse gas emission targets are met. The goal of coral reef restoration has therefore shifted toward recovering or maintaining key ecosystem processes, functions, and services through the next few decades of climate change, rather than restoring to a historical baseline.

Here, we suggest that the term "coral reef restoration" be used to describe an active intervention aimed to assist the recovery of reef structure, function, and key reef species in the face of rising climate and anthropogenic pressures, promoting reef resilience and the sustainable delivery of reef ecosystem services. These interventions include reducing impacts, remediation, and rehabilitating ecosystem function, following standards developed by SER (Gann et al., 2019, **Figure 1**). Actions aimed at protecting and enabling recovery (e.g., waste and water quality management) can be broadly categorized as "proactive," and they support "reactive" actions, commonly referred to as "restoration." These terms are meant to replace "passive" and "active" on the basis that "passive" has a negative connotation of implying that no action is necessary. "Reactive" actions are aimed at repairing ecosystem function and assisting the recovery of a degraded reef system, should it not be able to recover on its own (**Figure 1**).

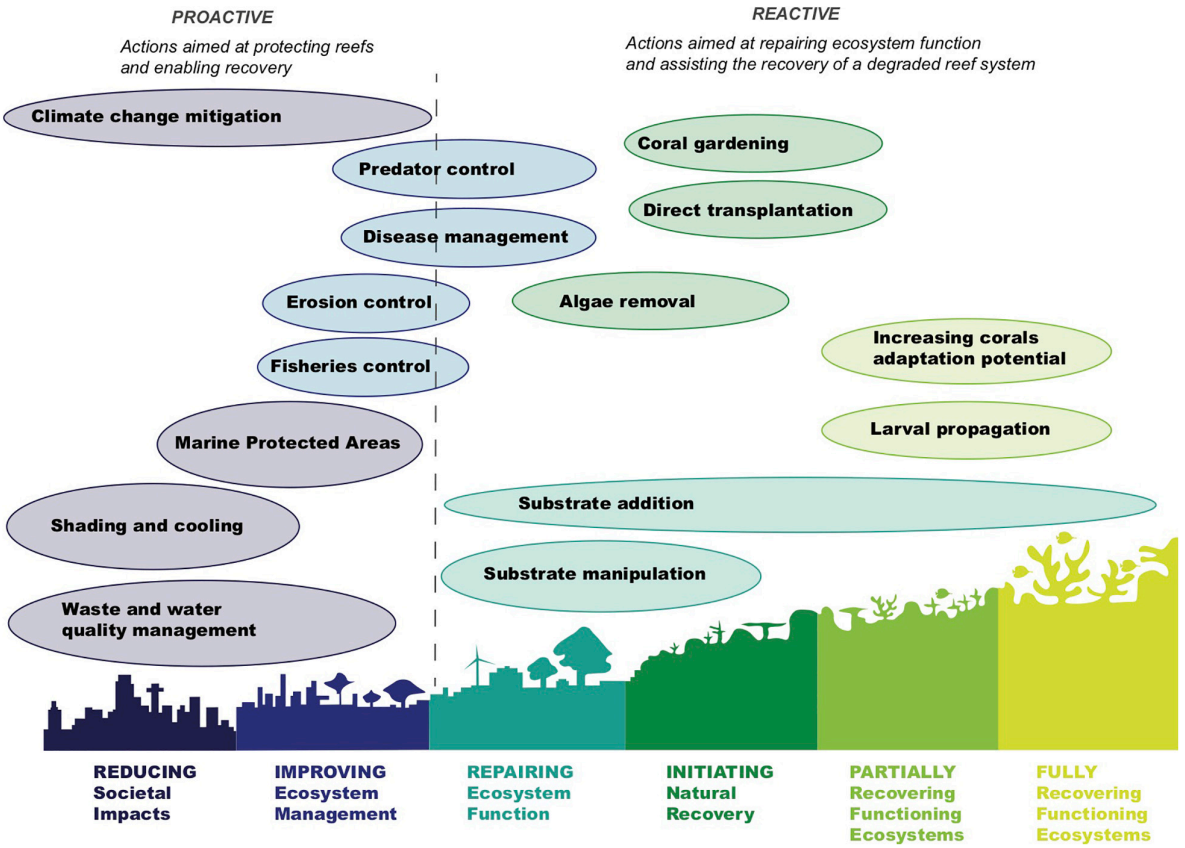

FIGURE 1 | Continuum of proactive and reactive interventions for coral reef conservation and restoration. Adapted from SER guidelines (Gann et al., 2019).

Restoring corals should never be the first point of action in a reef management strategy, but rather part of a strategy in a carefully planned ecosystem management framework (Edwards, 2010). Avoiding and mitigating local impacts to reefs should always be the priority, and restoration should never be used as an excuse to justify degradation in another area.

Goals of Coral Reef Restoration

Defining clear goals is critical to effective planning, implementation, and monitoring of restoration. In conservation, goals are commonly defined as the ultimate impact you hope to achieve by conducting interventions over the medium to long term (e.g., 5–20 years; Open Standards for the Practice of Conservation, Conservation Measures Partnerships (CMP), 2020). The overarching goal of most coral reef restoration projects is to recover a functioning and self-sustaining reef ecosystem, and coral reef restoration efforts should be planned as a long-term intervention. However, there are narrower, but still important goals that motivate managers and practitioners. Below is a list of common goals for coral reef restoration (**Table 1**).

These goals are non-exclusive and may often complement one another. However, in planning coral restoration, clearly articulating the project goal(s) should be the first action (Shaver et al., 2020). Then, objectives can be defined to track, and accomplish the goals over short time periods (e.g., 1–3 years). To manage ecosystems effectively, objectives should be Specific, Measurable, Achievable, Relevant, and Time-bound (SMART). Objectives should be informed by reference ecosystems but should consider future-anticipated environmental change (McDonald et al., 2016; Gann et al., 2019; Goergen et al., 2020). Examples of SMART objectives specific to coral reef restoration include: *XX genotypes from XX coral species outplanted on XX reefs in the first year resulting in XX% increase in genetic diversity*, or *XX increase in coral cover at XX site within 3 years resulting in XX% reduced wave action* (Shaver et al., 2020).

Current Methods of Coral Reef Restoration

Methods of coral reef restoration are evolving rapidly with investment in research and development. A number of emerging interventions are currently being tested experimentally across various scales, from individual corals (e.g., genetics, reproduction, physiology), to coral populations, reef communities, and ecosystems. The US National Academies of Science, Engineering, and Medicine (NASEM) and the Reef Restoration and Adaptation Program (RRAP) have recently provided an extensive review of a number of interventions that could increase the physiological resilience of corals to

TABLE 1 | Goals and associated rationales of coral reef restoration.

Goals	Rationales- use restoration to. . . .
Socio-economic goals	
a. Sustain or recover coastal protection	Sustain or re-establish the regulating ecosystem services provided by reefs to protect coastal communities and infrastructure by attenuating wave energy and mitigating disturbances such as erosion and coastal flooding
b. Sustain or recover fisheries production	Sustain or re-establish the provisioning services delivered by reefs in providing habitat and nursery areas for commercially important fisheries
c. Sustain or enhance local tourism opportunities	Maintain reef aesthetics to support local reef tourism and/or provide opportunities for eco-tourism experiences
d. Promote local coral reef stewardship	Support local communities and/or indigenous Traditional Owners to engage and reconnect with the local reef environment, improve reef custodianship and promote intrinsic value of reefs (spiritual, traditional, worship)
Ecological Goals	
a. Re-establish reef ecosystem function and structure	Rehabilitate the function, structure, diversity and health of degraded coral reef ecosystems
b. Mitigate population declines and preserve biodiversity	Assist the recovery of endangered coral populations, and preserve innate reef biodiversity from genes to phenotypes to ecosystems
Climate change mitigation and adaptation goals	
a. Mitigate impacts and promote reef resilience in the face of climate change	Support resistance and recovery processes to reduce risks of impact and ensure that reefs persist through current and projected changing climate conditions
Disturbance-driven goals	
a. Respond to acute disturbance to accelerate reef recovery	Assist natural recovery process when reefs are affected by acute disturbances such as storms, predator outbreaks, ship groundings, and other structural damages
b. Mitigate anticipated coral loss prior to disturbance	Adopt an effective "no net loss" mitigation policy whereby if a disturbance (e.g., coastal development) cannot be avoided, it should be minimized and offset for example by relocating anticipated ecological losses prior to disturbance

TABLE 2 | Current methods of coral reef restoration adapted from Boström-Einarsson et al. (2020).

Method	Definition
1. Direct transplantation	Transplanting coral colonies or fragments without an intermediate nursery phase.
2. Coral gardening	Transplanting coral colonies or fragments with an intermediate nursery phase. Nurseries can be *in situ* (in the ocean) or *ex situ* (flow through aquaria).
3. Substrate addition (artificial reef)	Adding artificial structures for purposes of coral reef restoration as a substrate for coral recruitment, coral planting, and/or for fish aggregation
3. 1 Electro-deposition	Adding artificial structures that are connected to an electrical current to accelerate mineral accretion.
3.2 Green engineering	Adding artificial structures designed to mimic natural processes and be integrated into reef landscapes (nature-based solutions, eco-designed structures, living shorelines).
4. Substrate manipulation	Manipulating reef substrates to facilitate recovery processes.
4.1 Substrate stabilization	Stabilizing substratum or removing unconsolidated rubble to facilitate coral recruitment or recovery.
4.2. Algae removal	Removing macroalgae to facilitate coral recruitment or recovery.
5. Larval propagation	Releasing coral larvae at a restoration site, after an intermediate collection and holding phase, which can be in the ocean or on land in flow through aquaria.
5.1 Deployment of inoculated substrate	Deploying settlement substrates that have been inoculated with coral larvae.
5.2. Larval release	Releasing larvae directly at a restoration site

climate change (Bay et al., 2019; National Academies of Sciences Engineering and Medicine (NASEM), 2019). The 23 intervention types investigated by NASEM include novel approaches such as cryopreservation, managed relocation of corals to promote assisted gene flow (AGF), or microbiome manipulations (National Academies of Sciences Engineering and Medicine (NASEM), 2019). The Reef Restoration and Adaptation Program (RRAP) in Australia is evaluating "moonshot" solutions that can operate across the entire scale of the Great Barrier Reef, including assisting the evolutionary adaptation of reef species to warmer waters, and mass production and release of coral larvae to seed reefs (Bay et al., 2019). Other field experiments are underway in places like Fiji and Kiribati to facilitate natural processes of reef recovery by capitalizing on innate reef resilience (Coral for Conservation, 2020). There, the focus is on using colonies that have survived recent episodes of coral bleaching as well as encouraging ecological synergies by actively removing coral predators and re-introducing fish and sea urchins to control macro-algae overgrowth (Coral for Conservation, 2020). These proposed interventions represent a substantial body of research and potential for improving reef restoration, yet most are still in the research and development phase, and may take years before becoming feasible for large-scale implementation.

In contrast, five coral reef restoration methods have already been widely applied and tested in the field (**Table 2**). Some are more widely used than others. For example, a recent review by Boström-Einarsson et al. (2020) found that the majority of documented projects (almost 70%) involved coral planting (e.g., direct transplantation, coral gardening). Other methods are far less popular, for example substrate manipulation methods comprised only 10% of all projects, and larval propagation 1% of all projects (Boström-Einarsson et al., 2020).

THE VALUE OF CORAL REEF RESTORATION IN THE FACE OF RISING ENVIRONMENTAL CHALLENGES

The Global Climate Change Challenge

Clearly the biggest obstacles to natural recovery of coral populations are global climate change and associated mass coral bleaching. Even if global targets set by the Paris Agreement

are met in the future, current greenhouse gas emissions are still increasing, and the increase in frequency of mass-bleaching events in the last 5 years suggest that coral reefs globally are very close to their temperature limits (Hughes et al., 2018). In this context, some scientists argue that active interventions, such as reef restoration, do not address the underlying causes of reef declines (Bruno and Valdivia, 2016; Hughes et al., 2017; Bellwood et al., 2019). Coral reef restoration has been criticized as an expensive, temporary fix that is not deployable at scales that match the scale of disturbances, and a distraction from other conservation strategies that are more focused on addressing the root causes of disturbances (Bellwood et al., 2019; Morrison et al., 2020). However, it is important to differentiate among the portfolio of actions available to tackle climate change and to ensure coral reefs ecosystems and their associated services can persist in the future. Coral reef restoration is not designed to reduce climate impacts, but rather is intended as a complementary tool to support natural recovery following disturbance in key areas. Given the many uncertainties associated with different climate scenarios (Bindoff et al., 2019), the key challenge is to design coral restoration efforts such that the realities of climate change are embedded in the choice of goals, objectives, and methods (Shaver et al., 2020). It is not an "either or" situation, as climate change mitigation does not preclude investment in local management strategies designed to build the resilience and adaptation of the socio-ecological coral reef systems.

Further exacerbating the situation are local causes of reef degradation. Identifying, reducing, and/or removing these local pressures are all critical steps in effective coral reef restoration (Edwards, 2010). There is no point replanting a coral reef where corals have died due to poor water quality if water quality has not been addressed and improved prior to planting. It is also not worth the valuable and limited resources of most local reef managers to undertake restoration if the reef can recover without restoration efforts, which can happen on reefs where coral recruitment is not limited and if there is enough time between predicted disturbance events. If, on the other hand, there is a barrier to recovery that cannot be overcome naturally, then restoration is necessary to kick start system recovery.

Barriers to Natural Recovery

The most common, non-climate related, barriers to natural recovery are substrate limitations and/or recruitment limitations. Substrate limitation refers to instability and suitability, which both affect the capacity of coral larvae to recruit, settle, and grow. For example, unconsolidated coral rubble impedes coral attachment and may create further physical damage (Ceccarelli et al., 2020), while substrate covered in macroalgae impedes coral settlement (Dixon et al., 2014). Recruitment limitation occurs when the supply of coral larvae (or fragments) from reproductive adult populations is exceedingly low or when a reef is disconnected from larval supply. Finally, physiological barriers to natural recovery have emerged in places where coral growth and survival have become limited by new thermal extremes (Schoepf et al., 2015; Thomas et al., 2018).

Restoration as a Call to Action

There is a growing argument that the risk of doing nothing far outweighs the risks or uncertainties of active interventions (Anthony et al., 2017, 2020). The rapid increase in implementation of coral reef restoration strategies is driven by a sense of urgency following catastrophic loss in global coral cover in the last decade. This sense of urgency creates unique scientific uncertainties as there is not enough time to wait for climate action to be enacted, for pressures to stop, or for repeated experimental methods to be published in scientific journals before action is taken. Even in the context of continued coral declines attributed to climate change, goals outlined in **Table 1** highlight the varied motives for coral reef restoration across socio-ecological scales. At local scales, and in the short-term, coral reef restoration can provide benefits such as: (1) increasing genetic diversity and thus the potential for adaptation, (2) helping to prevent the extinction of some species, (3) assisting species migration to new locations, (4) continuing to provide critical ecosystem services, and (5) providing tangible mechanisms for people to combat ecological grief. Importantly, coral reef restoration should not be considered as a solution on its own but rather as part of an integrated resilience-based management framework (e.g., McLeod E. et al., 2019) that includes a hierarchical portfolio of actions from threat reduction (i.e., climate change mitigation, water quality controls, fishing regulations), to actions that support the recovery and resistance of ecosystem processes such as marine protected areas or coral predator removal (e.g., crown-of-thorns starfish) as illustrated in **Figure 1**. As such, coral reef restoration may span beyond planting scleractinian corals to include interventions such as algae removal and fish introduction that support the recovery of reef function. Also, within that framework, the different strategies integrate both social and ecological adaptive capacity to manage for uncertainty and change (McLeod E. et al., 2019). Coral reef restoration can be a useful tool to support resilience, and if well integrated into a resilience-based management framework, can play a key role in meeting Sustainable Development Goals associated with the UN Decade on Ecosystem Restoration (Claudet et al., 2019). Nonetheless, implementation of coral reef restoration actions should not be haphazard and should not divert resources away from other reef management strategies that actively control stressors. Integrating investments for coral reef restoration within funding for resilience-based management may help maximize the positive impacts of current and future strategies.

RECOMMENDATIONS

Restoration is only one in a suite of intervention options available to reef managers. Reef restoration should always be undertaken in concert with complementary strategies and integrated in a resilience-based management framework (Hein et al., 2020a, UNEP). Also, restoration might not always be appropriate. The following considerations, should be made prior to planning and designing: (1) assess the cause(s) of coral decline (e.g., pollution, human activities, bleaching); (2) review factors affecting the potential for natural recovery of corals (e.g., spawning capacity,

barriers to coral recruitment, limits to coral growth); and (3) determine which intervention is best suited under the circumstances to achieve the stated goals of the restoration project (Edwards, 2010; Hein et al., 2020a, UNEP). These steps will help identify (a) whether coral reef restoration is necessary, and (b) what might need to done beforehand (e.g., improving water quality, improving the physical integrity of reef substrate, or recovering key ecological processes (Edwards, 2010; Hein et al., 2020a, UNEP).

Planning and Design

Restoration is not a "one size fits all" approach, and each aspect of a restoration program, from goals to methods used, should be tailored to the specific needs and abilities of each location. Key elements of effective and efficient designs include: (1) defining SMART goals and objectives, (2) developing a climate-smart, adaptive strategy, and (3) engaging stakeholders early (Shaver et al., 2020). Pilot studies should be included to refine the choices of sites and methods and the overall action plan prior to full implementation (Shaver et al., 2020). In addition, current information and projections on the specific vulnerability of a reef site to climate change should be incorporated in initial planning to ensure the chosen intervention(s) have a chance to withstand future conditions (West et al., 2017, 2018; Shaver et al., 2020). Engaging with stakeholders, local communities, indigenous communities, and traditional owners in all stages of restoration planning and implementation is critical to reduce potential conflicts associated with the use of reef resources and to maximize collaborations and investment opportunities (Gann et al., 2019; DeAngelis et al., 2020). Incorporating traditional or local knowledge of the specific reef system of concern will improve the chances of restoration success. Appropriate engagement and communication are critical to maximize the flow of socio-cultural and economic benefits beyond the people directly involved in the restoration effort, therefore securing longer-term support. Coral reef restoration can be a useful educational tool that encourages tangible behavioral changes and improves the social resilience of local communities, the economic resilience of local reef-reliant industries, as well as the ecological resilience of the reef (Hein et al., 2019).

Monitoring and Communication

Appropriate monitoring of coral reef restoration efforts should assess outcomes against initial goals and objectives at appropriate time scales. Monitoring is crucial to inform and facilitate adaptive management, and to increase transparency and accountability. Ideally, restoration efforts should be set up in a way that allows for an assessment of effectiveness with control sites and/or following a before/after/control/impact (BACI) design (see Falk et al., 2006; Gann et al., 2019; Goergen et al., 2020), and monitored and evaluated consistently (Pioch et al., 2017), so improvements can be made as the project evolves and environmental conditions change. Comparing outcomes across projects will necessitate a standardization of monitoring protocols across socio-ecological dimensions (Hein et al., 2017; Goergen et al., 2020). Systematically monitoring a few metrics (e.g., dimension of restored area, genotypic diversity, coral population abundance) as outlined in Goergen et al. (2020) is also important to further the understanding of the effectiveness of coral reef restoration to assist the recovery of degraded reefs. Monitoring outcomes also need to be better communicated to improve collaboration and outreach (DeAngelis et al., 2020). Within a project community, it is important to communicate often to keep the public engaged and to use non-scientific language that is easily understandable and relevant to target audiences. Communication among managers and practitioners is also important to share successes, failures, and foster collaborations to advance the field.

Restoration Goals

Defining specific goals and objectives will help managers and practitioners develop targeted monitoring plans and enhance the clarity of reporting on the outcomes of their project(s). In many instances, project(s) will tackle more than one goal at a time and accrue multiple benefits as a result. However, each goal comes with specific challenges. The tables and figures below are provided to help cross reference goals, methods, and other relevant factors. In **Table 3** we provide key considerations for various restoration goals. For example, goals associated with sustaining tourism may be accomplished in relatively short time frames (<3 years) if tourism operators are involved in the project early-on, with clear communication plan and sustainable funding schemes (**Table 3**). Projects attending to acute disturbances require effective emergency management plans to succeed in a short time frame. On the longer end of the spectrum, re-establishing a self-sustaining, functioning reef ecosystem is a more complex, longer-term goal that depends upon other ecological variables (e.g., water quality, genetic diversity of corals). Choosing goals should be done thoughtfully and with respect not only to the environmental challenges but with respect to the capacity of management (e.g., sustainable funding, interest, personnel).

Method(s) Selection

There are a growing number of methods for coral reef restoration and selecting a method should be done with careful consideration of the projects' goals.

Method(s) selection should be driven by specific goals the coral restoration efforts are designed to achieve. An index matrix prepared by experts in the field informs the suitability of each currently established methods for a particular goal (**Figure 2**). There, methods were ranked from least to most appropriate in fulfilling specific goals, based on the best-available current knowledge. For example, larval release and the deployment of inoculated substrates were ranked as most appropriate for the goal of mitigating population decline and preserving biodiversity (**Figure 2**), on the basis that these two methods will maximize genetic diversity at the restored site(s). Note that for most projects, multiple methods may be used to satisfy specific goals and associated objectives. For example, for the goal of responding to acute disturbances to accelerate recovery, both methods of direct transplantation and substrate stabilization were identified as most appropriate (**Figure 2**). Location and project specific characteristics should guide the choice of methods

TABLE 3 | Key considerations for applying coral reef restoration to satisfy specific goals.

Goals ►	**Socio-economic goals**			
Sub-goals ►	**a. Recover and sustain coastal protection**	**b. Recover and sustain fisheries production**	**c. Sustain local tourism opportunities**	**d. Promote local coral reef stewardship**
Timeframe	Medium (3–5 years)	Long (>5 years)	Short (<3 years)	Short (<3 years)
Key considerations	- Use nature-based solutions (green engineering, eco-design, biomimetics) as much as possible - Careful consideration of hydrology in site selection - Functional design should include ecological and physical function (habitat, species) - Consult with engineers so designs are robust (durable) against future disturbances and eco-friendly - Embed with coastal protection policies	- Site selection should consider fisheries protection and connectivity to healthy fish population - Design should maximize complexity and diversity of substrates - Design should consider potential for recruitment of desirable species - Engage fishermen and local communities as early as possible	- Engage the tourism industry in the project as early as possible - Develop effective communication plan - Design should incorporate aesthetics considerations - Develop specific training to reduce risks of doing more harm than good - Follow sustainable funding models	- Engage local stakeholders in the project as early as possible -Incorporate indigenous knowledge is site selection and project design -Target young people - Develop effective communication plan - Embed within Resilience Based Management frameworks
Goals ►	**Ecological goals**		**Climate adaptation and support goals**	
Sub-goals ►	**a. Re-establish reef ecosystem function and structure**	**b. Mitigate population declines and preserve biodiversity**	**c. Mitigate impacts and promote reef resilience through climate change**	
Timeframe	Long (>5 years)	Medium (3–5 years)	Medium (3–5 years)	
Key considerations	- Long-term process - Integrate within Resilience-Based Management frameworks - Maximize diversity and functional redundancy from genotypes, to species, and growth forms - Consider positive ecological feedbacks beyond coral transplantation	- Careful site selection where disturbances have been mitigated - *In situ* and *ex situ* nurseries can be used as gene banks for endangered species - Maximize genetic diversity especially when target specific species	- Site selection and project design based on climate smart models - Species selection based on local knowledge of resilient coral assemblages and functional redundancy - Integrate research on coral adaptation mechanisms	
Goals ►	**Disturbance-driven goals**			
Sub-goals ►	**a. Respond to acute disturbance to accelerate reef-recovery**	**b. Mitigate anticipated coral loss prior to disturbance**		
Timeframe	Short (<3 years)	Short (<3 years)		
Key considerations	- Stabilize substrate and immediate triage of live corals - Mitigate source of disturbance prior to restoring - Have an emergency response plan in place ahead of time (similar to oil spill response planning) - Might be constrained by insurance and permitting rules	- If possible, move corals to *in situ* or *ex situ* nurseries prior to disturbance - Relocation site should have similar environmental parameters than donor site - Mitigating the disturbance to avoid relocation is always the favored solution - Aim for "no-net loss" to offset ecological losses		

further (Shaver et al., 2020). Interestingly, for many of the goals (e.g., recover and sustain coastal protection, recover and sustain fisheries production), none of the current methods were ranked as "most appropriate," further highlighting some critical gaps between the goals and current methods for coral reef restoration. However, given the fast pace at which the field of coral reef restoration is expanding and the increasing level of investment, new methods that may be more appropriate are in development.

Providing guidance on how and when to use various methods of restoration was part of the driving force behind the UNEP Report (Hein et al., 2020a, UNEP). Each of the established coral reef restoration methods comes with its own set of benefits and challenges. The rationale behind selecting one method over another is generally not reported in the literature. The lack of guidance is likely due, again, to a lack of monitoring and reporting of long-term outcomes of coral reef restoration efforts (Hein et al., 2017; Boström-Einarsson et al., 2020), but also to a

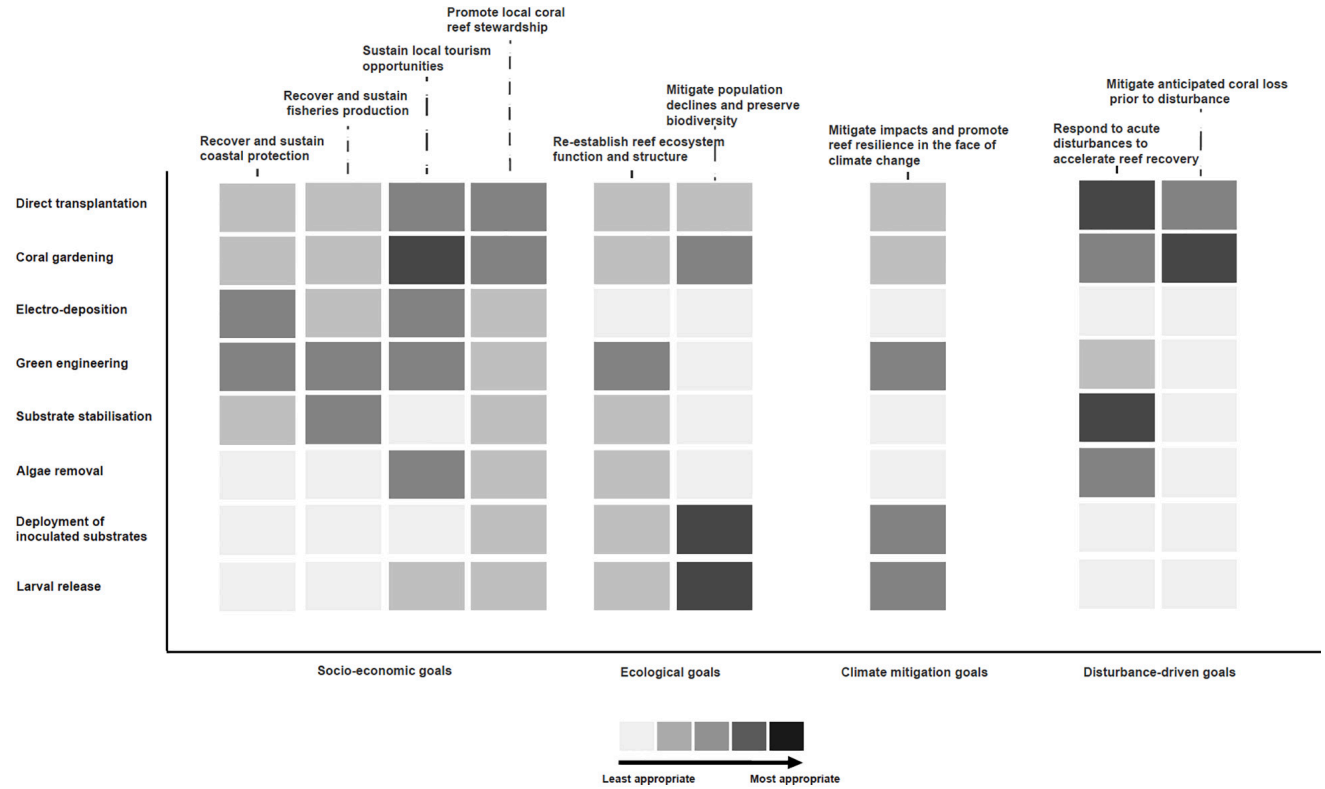

FIGURE 2 | Method suitability matrix for each coral reef restoration goals. The darker the color, the more appropriate a method is to each specific goal. Note that for many goals multiple methods may be suitable.

lack of studies that compare outcomes from different coral reef restoration methods (Hein et al., 2020b). Many different criteria may be considered when selecting one type of intervention over another, many of which will be location- and project-specific (Shaver et al., 2020). For example, one might consider the flexibility of a method in terms of the ease of implementing and adjusting the effort to adapt to unforeseen disturbances; others might be driven by externalities associated with permit requirements such as ensuring local communities can actively be involved in the restoration process. Three criteria: cost, efficiency, and scalability are particularly important driving forces of that decision-making process.

Cost, Efficiency, and Scalability

Eleven coral reef restoration experts assessed each of the most established coral reef restoration methods. Experts were selected from the ICRI *ad hoc* committee on coral reef restoration as well as from the CRC leadership team and ranged from academics, to managers, and practitioners from various reef regions around the globe. Scores were provided for three criteria: cost, efficiency and scalability, providing a qualitative comparison among methods (**Figure 3**). Results, presented as violin plots, help identify consensus and variability among the experts' opinions and display variability in the responses. For example, there was consensus on the high cost of substrate addition methods, but high variability on the efficiency and scalability of this method. Electro-deposition ranked as the least efficient and scalable, and among the costliest methods (**Figure 3**). There was high variability in the scores overall—most plots spanning almost the whole range from 0 to 10 (**Figure 3**), which is likely due to the lack of rigorous monitoring and the limited implementation of some of the methods (e.g., larval restoration, Boström-Einarsson et al., 2020). With appropriate monitoring (as suggested by Goergen et al., 2020), estimates of cost-effectiveness and scalability could improve given increasing investment in coral reef restoration. However, for most methods, the overall trend of high costs but medium to low efficiencies (**Figure 3**. The discrepancies of opinions among experts for most metrics also reflect the relative youth of coral reef restoration science and highlight the future opportunities for innovations and solutions that are more scalable, affordable and effective building upon the body of work and experiences gained in the field to date.

Challenges and recommendations for each of method are highlighted in **Table 4**. While not prescriptive, **Table 4** is intended to provide guidance, beyond the suitability of methods to goals outlined in **Figure 2**, and the relative cost, efficiency, and scalability illustrated in **Figure 3**. For example a group interested in restoring a reef for the goals of "preserving biodiversity" as well as "sustaining local tourism opportunities," may choose to combine at least two methods- larval propagation methods would help ensure long-term coral genetic variation and potential for

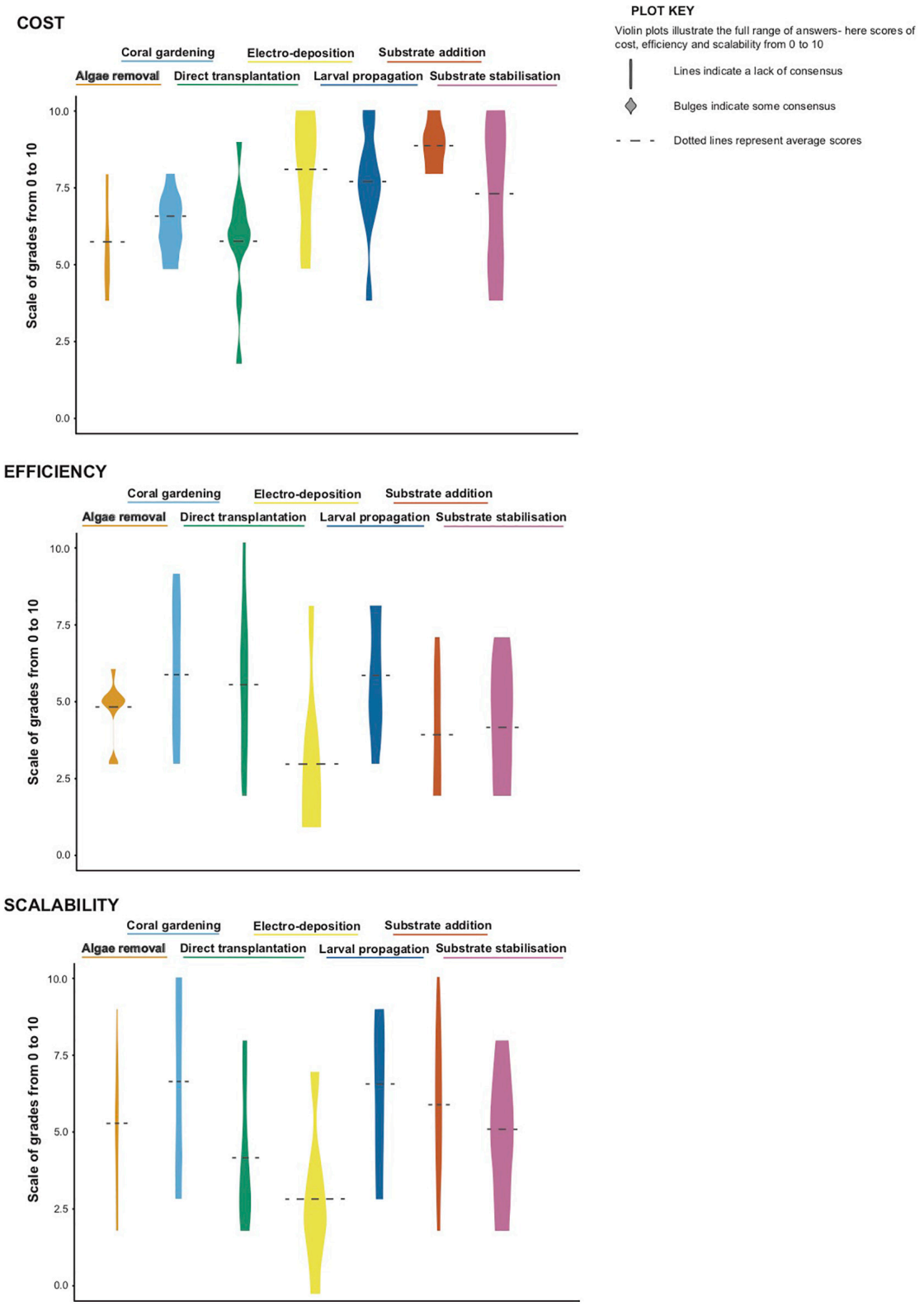

FIGURE 3 | Violin plots representing cost, effectiveness, and scalability of seven common coral reef restoration methods, graded on a scale of 0–10 by $n = 11$ global experts

TABLE 4 | Specific challenges and recommendations for each of the currently established methods of coral reef restoration.

Methods ▼		Challenges	Recommendations
1. Direct transplantation		- Can be expensive - Availability of diverse coral fragments as donor material - Limited to small scale projects	- Planting sites should be as similar to donor site as possible - Avoid planting during storm and bleaching season - Maximize diversity of fragments as much as possible - Attachment methods: invest time, use non-toxic materials and/or chemicals - Use citizen science to reduce cost and increase engagement - Plan to monitor and maintain outplanting site
2. Coral gardening		- Cost and labor intensive - Limited to small scale projects - Material used are often not eco-friendly or not resistant to damage or degradation over time - Health of corals can be compromised due to algae overgrowth and spread of disease in high density nurseries - Requires sustained maintenance that can be expensive	- Carefully consider depth and other environmental factors (e.g., water quality, wave action) at nursery sites - Plan for extreme weather events - Plan to maximize diversity of fragments in nursery- growth forms, sources, genetic - Two-step process: see recommendations for direct transplantation - Plan for long-term maintenance and removal of the nursery once restoration project is complete
3. Substrate addition (artificial structures)	**3.1 Electro-deposition**	- Very expensive and difficult to deploy - Limited evidence of success - Needs a reliable power source	- Develop more research to justify its usefulness compared to simpler structures - Consider alternative local sources of energy (solar, wind)
	3.2 Green engineering (Nature Base Solution, eco-design)	- Expensive to design and deploy - Limited to small scale projects - Limited evidence of success linked to structures being overgrown by corals - Failure can have lasting detrimental effect on reef aesthetics (e.g., concrete blocks)	- Consult engineers for optimal design depending on goals - Materials should become living structures (recruitment potential on the structure following bio-mimetic principles of green engineering) - Consider impact of structure(s) on the site hydrodynamics, and aesthetics - Mostly relevant when reef structure and stability has been compromised
4. Substrate manipulation	**4.1 Substrate stabilization**	- Can be very expensive to deploy - Can have poor aesthetics - Limited evidence of success- approaches not very well documented - Difficult to assess when it's appropriate to use (natural recovery versus intervention)	- More research into natural ways to stabilize substrate (e.g., natural binding by sponges or crustose coralline algae) - Apply careful consideration of hydrodynamics
	4.2 Algae removal	- Algae can grow back quickly - Very labor intensive - Risk of removing natural, non-invasive algae species and disrupt positive ecological processes	- Use in conjunction with other intervention that increase herbivory and control water quality - Time removal around coral recruitment - Use citizen science and volunteers to reduce and maximize engagement
5. Larval propagation	**5.1 Deployment of inoculated substrate**	- Expensive, labor intensive, and requires expert knowledge - Limited evidence of long-term success due to the novelty of the method - Substrates can become overgrown by algae, sponges, and other sessile invertebrates compromising recruits' health and survival	- Need to improve coral recruits' growth and survival substrates - Invest in technology development and training to scale-up current efforts - Optimize outplanting strategy to promote self-sustaining populations of sexual recruits
	5.2 Larvae release	- Expensive- requires a lot of equipment and involvement of experts - Difficult to engage the public and community members - Evidence of success currently limited by high post-settlement mortality - Timing of action dictated by coral spawning - Long time scale for meaningful ecological outcomes	- Consider mixing genets from different regions (Assisted Gene Flow) - Potentially one of the most scalable methods for coral reef restoration, and a research priority for making this method more accessible and improving coral recruits health, growth, and survival

adaption, while coral gardening could engage local tourists and create a sustainable funding mechanism. Another group may want to increase fisheries productions while protecting their coastline. This group may use artificial substrate to protect their coastline, and plant branching coral from a nearby nursery (or coral garden) on the substrate to provide fish with complex habitat. If these methods are too costly, substrate stabilization and direct transplantation of corals of opportunity could be substituted. We hope the series of tables and figures provided here are a helpful guide to thinking through the various goals and

methods of restoration, which vary widely depending on local environmental condition, available capacity, and funding.

CONCLUSION AND RECOMMENDATIONS

The need for restoration is accelerating as coral reefs around the world continue to experience catastrophic declines in coral health and cover. One of the roles of the UNEP is to provide expert guidance on how coral reef restoration interventions may be used to protect and enhance the delivery of reef ecosystem services in the future. In this synthesis, several key recommendations emerge. First, it is important to recognize that coral reef restoration is not a "silver bullet" designed to address the rising threats of climate change and anthropogenic disturbances. It should never be used as an excuse to justify reef degradation. Second, coral reef restoration can be a useful tool to support resilience, especially at local scales where coral recruitment is limited, and disturbances can be mitigated. Third, coral reef restoration interventions should be integrated within a resilience management framework, as a continuum of reactive and proactive actions, focusing not just on restoring hard corals but the overall function of the reef community. Fourth, monitoring of appropriate metrics over time is essential so that management decisions can be more scientifically robust. Finally, applying coral reef restoration methods effectively and efficiently requires "climate-smart" designs that account for future uncertainties and changes (Parker et al., 2017; West et al., 2017, 2018). Current information and projections on the specific vulnerability of a reef site to climate change should be incorporated in initial planning to ensure the chosen intervention(s) have a chance to withstand future conditions (Van Hooidonk et al., 2016; Shaver et al., 2020).

Following recommendations from the Society for Ecological Restoration, we suggest coral reef restoration strategies follow four critical directions: (1) planning and assessing around specific goals and objectives, (2) identifying adaptive strategies to balance risks and trade-offs, (3) engaging communities in all stages of the restoration efforts, (4) developing long-term monitoring plans to allow for adaptive management and improving the understanding of methods' effectiveness for specific goals. With ongoing and further investment in research and development, the cost-effectiveness of established and new methods should improve the scalability and effectiveness of coral reef restoration interventions. Supporting such investment is critical to improving the capacity to intervene locally and globally and improve the chances for coral reefs to thrive into the future.

AUTHOR CONTRIBUTIONS

MH, IM, ES, TV, SP, LB-E, MA, and GG conceived the manuscript and reviewed and edited the manuscript. MH, IM, TV, and GG wrote the manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Marine Science

BRIEF RESEARCH REPORT
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Differential Effects of Substrate Type and Genet on Growth of Microfragments of *Acropora palmata*

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Global decline of coral reefs has led to a widespread adoption of asexual propagation techniques for coral restoration, whereby coral colonies are fragmented and allowed to re-grow before being returned to the reef. While this approach has become increasingly popular and successful, many questions remain regarding best practices to maximize restoration speed, efficiency, and survival. Two variables that may influence growth and survival of asexually fragmented colonies include coral genet and growth substrate. Here, we evaluate the effects of genet and substrate (commercially available ceramic vs. in-house made cement) on the survival and growth of 221 microfragments of elkhorn coral *Acropora palmata* over 193 days. All corals survived the experimental period, and doubled their initial size in 45 days, with an average growth of 545% over the study duration. Growth was generally linear, though the growth of some corals more closely matched logistic, logarithmic, or exponential curves. Both genet and substrate had significant effects on coral growth, though the two factors did not interact. Genet had a stronger influence on coral growth than substrate, with the fastest genet growing at 216% the rate of the slowest genet. Corals on cement substrate grew at 111.9% the rate of those grown on ceramic. This represents both a significant cost savings and elimination of logistical challenges to restoration practitioners, as the cement substrate ingredients are cheap and globally available. Our work shows that both genet and substrate should be considered when undertaking asexual restoration of *Acropora palmata* to maximize restoration speed and efficiency.

Keywords: *Acropora*, substrate, genotype, coral restoration, microfragmentation, asexual propagation

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INTRODUCTION

Coral reefs, often characterized as underwater rainforests, are among the most biodiverse ecosystems on Earth (Reaka-Kudla, 1997; Jaap, 2000) and perform critical ecological and economic functions for millions worldwide (e.g., Moberg and Folke, 1999; Ferrario et al., 2014). However, dramatic declines in coral cover globally over the past several decades (Gardner et al., 2003; Côté et al., 2005; De'ath et al., 2012) have eroded the functionality of these ecosystems and gravely threaten them and the services they provide (Jones et al., 2004; Alvarez-Filip et al., 2011). These declines have been driven by both natural and anthropogenic stressors such as overfishing, disease, storms, nutrient pollution, coastal development, and climate change (e.g., Gladfelter, 1982; Hughes, 1994; Bruno et al., 2007; Burke et al., 2011; Walton et al., 2018). Reefs will continue to decline as

stressors such as bleaching, ocean acidification, and overall impacts of climate change become more prevalent (Hoegh-Guldberg et al., 2007; Albright et al., 2010; Hughes et al., 2017).

The Florida Reef Tract (FRT), located at the southern terminus of the Florida Peninsula spanning from the Florida Keys up the eastern part of the state to Martin County, is the third largest barrier coral reef on Earth and the only barrier coral reef in the continental United States. As of 2015, the Florida Keys National Marine Sanctuary located within the FRT produced an estimated $4.2 billion in economic output to the state of Florida and attracts over three million visitors each year (Tbd Economics, LLC, 2019). However, like most Caribbean reef systems, the FRT has suffered dramatic declines in coral cover from an estimated 25% in the 1980s to an average stony coral cover of 6% today (Ruzicka et al., 2013). Among the several dozen coral species that have dominated shallow water reefs in the western Atlantic for hundreds of thousands of years, elkhorn coral, *Acropora palmata,* was historically among the most abundant (Jackson, 1992; Precht and Miller, 2007). However, like other coral species, *A. palmata* has declined dramatically in Florida over recent decades. This decline, driven largely by outbreaks of white-band disease in the 1970s (Gladfelter, 1982; Aronson and Precht, 2001) and more recently by additional stressors including ocean warming, human activities, and storm damage (Baums et al., 2003; Burke et al., 2011; Williams and Miller, 2012; Williams et al., 2017), led to *A. palmata* being listed as critically threatened by the IUCN in 2008 (Aronson et al., 2008). No significant signs of recovery have been recorded (Miller et al., 2002; Johnson et al., 2011), and recent data suggest that *A. palmata* populations are now too sparsely distributed across the reef tract to successfully reproduce without direct intervention (Knowlton, 2001; Williams et al., 2008; Miller et al., 2018; National Academies of Sciences Engineering Medicine, 2019). More broadly, because no other Caribbean corals closely resemble *A. palmata* morphologically or ecologically, the only way in which Caribbean coral reefs could regain their architectural composition and structural and biological function would be to restore *A. palmata* specifically (Chamberland et al., 2015). Because species such as *A. palmata* have experienced relatively rapid declines over the last several decades (Bruckner, 2002; Miller et al., 2002; Sutherland and Ritchie, 2004), it is important for practitioners to grow corals quickly and efficiently to reach goals of population enhancement.

The dire state of coral reefs worldwide has led to a surge in active coral restoration efforts. These efforts, which have become increasingly effective (Young et al., 2012; Carne et al., 2016), provide a critical capability to help coral reefs survive the Anthropocene until the drivers of coral loss can be directly addressed. Generally, coral restoration efforts are classified either as sexual restoration, where individual genets are fertilized, grown, and released (or "outplanted") into the wild, or asexual restoration, in which larger colonies are broken into clonal sub-fragments. Though hobbyists have been asexually fragmenting aquarium corals for many decades, the asexual restoration approach known as "coral gardening" has only been in use for a quarter century (Rinkevich, 1995). This approach, which involves raising corals in nurseries before 'outplanting' them back onto reefs, is practiced worldwide (e.g., Montoya-Maya et al., 2016), and includes over 150 programs in more than 20 countries in just the Caribbean and Western Atlantic alone (Lirman and Schopmeyer, 2016). Coral gardening has reached ecologically meaningful scales with 10,000s of coral grown in nurseries and outplanted each year (Lirman and Schopmeyer, 2016). To date, most restoration practitioners focus on asexual coral production through fragmentation (fragment size of several to many cm^2) or microfragmentation (fragment size of $\sim$1 cm^2) in part because it is relatively straight-forward in methodology, inexpensive, and can produce large amounts of biomass within short periods of time thus increasing the capability of restoring reefs at large scales. Another advantage of asexual restoration is the capability to propagate "winning" genets at large scales, maximizing the probability of restoration success and reef persistence.

Despite the large number of coral restoration practitioners now operating worldwide, and the environmental and social benefits of sharing best practices, and specific coral propagation methods are often poorly documented. Indeed, such methods often remain unpublished, or are relegated to hobbyist forums or gray literature (Barton et al., 2017), hampering effective, evidence based coral restoration (Boström-Einarsson et al., 2020). Accordingly, the publishing of best practices, failures, benchmarks, and even raw restoration data has been identified as a critical need to help develop the rapidly evolving field of coral restoration (Lirman and Schopmeyer, 2016; Boström-Einarsson et al., 2020).

There are many questions that remain regarding the best practices of coral restoration; among those are identifying how genet and substrate type alter the growth and survival of microfragments. Identifying superior growth substrates is critical for scaling up production at restoration facilities while keeping costs low. Similarly, understanding the various strengths and weaknesses of genets (including growth potential, resilience to disturbance, and reproductive potential) is particularly critical for asexual propagation programs, which often propagate fragments from a small number of genets in their nurseries. As new genets are obtained, practitioners may need to evaluate the characteristics of these genets (e.g., growth rate, stress tolerance) against those in the collection to prioritize propagation and outplanting. While genetic difference in growth across other species has been documented (Osinga et al., 2011; Lirman et al., 2014; Drury et al., 2017), the effects of genet or substrate on the growth rate of *A. palmata* in restoration facilities are unknown. To help fill this knowledge gap, we performed an experiment to determine the effect of substrate, genet, and their interaction on the growth and survival of *A. palmata* microfragments during the first six months of life following fragmentation. We hypothesized that coral will grow faster on commercially available ceramic disks than cheaper in-house made cement disks, and that growth rate would significantly differ among genets.

MATERIALS AND METHODS

Coral Housing

We housed the experimental corals in an outdoor 681 L flow through system (raceway) under a constant flow of

2.5 L/min drawn from a 24 m well. Prior to entering the raceway, water passed through a forced draft degasifier (R&B Aquatic Distribution, Inc., TX, United States), was aerated in a holding tank and mechanically filtered through sand. Water was further aerated within raceways using an air wand (Pentair plc, Minneapolis, MS, United States). We tested water quality twice daily with a YSI Professional Plus handheld multiparameter meter (Yellow Springs, OH, United States). Water remained within the restoration facility's optimal zones (~26–28°C, ~7.7–8.0 pH, ~38 psu). We used permanent overhead shade cloth (~67% light reduction), with additional plastic UV lids covered with ~67% shadecloth (added daily from 12:30 pm to 7:30 am) to maintain raceway temperature and reduce afternoon sunlight. Photosynthetically Active Radiation under the shade cloth was ambient; corals were exposed to a monthly average maximum of 186 μMol/m^2/s (January) to 520 μMol/m^2/s (July). To reduce fouling and overgrowth by filamentous algae, we added *Lithopoma tectum* snails as grazers.

Coral Fragmentation and Mounting

In June 2019, we microfragmented 22 ramets from four genets of *A. palmata* to create the 221 replicate colonies for the experiment (**Supplementary Table 1**). All ramets were captive, having been settled and raised at Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research and Restoration in Summerland Key, FL, United States. Corals had been raised within the common garden land-based nursery since fertilization in 2013 and 2014.

We cut each original ramet, measuring ~7 cm^2 in size, using a wet C40 diamond band saw (Gryphon Corporation, CA, United States) into microfragments averaging 0.57 ± 0.12 cm^2 SD (range 0.32–0.91 cm^2) using previously described methods (see Page et al., 2018). We then used cyanoacrylate glue (Bulk Reef Supply, MN, United States) to adhere the microfragments to one of two substrates: a commercially available ~7 cm^2 ceramic plug (Boston Aqua Farms, MA, United States), or a similarly sized concrete composite plug made in-house from a 3:12:7 ratio mix of commercially available playground sand, portland cement, and fresh water set in a rubber mold (see **Supplementary Material** for plug construction specifics and **Supplementary Figure 1**). Finally, we labeled the bases where microfragments were glued (hereafter "plugs") with unique identifiers and placed them on plastic racks in haphazard orientation. We held racks in a shaded recovery raceway for 3–4 days to stabilize post-fragmentation, then moved the corals to experimental raceways.

Data Collection and Analysis

We assessed coral growth by analyzing photographs taken at *c.a.* 2-week intervals in the photo analysis program ImageJ (Schindelin et al., 2012). Each photo included a scale bar, which was used in conjunction with the trace tool to calculate total coral area (**Figure 1**). Photos were analyzed by two trained observers; in the rare case the area calculations were not within 5% agreement, a third observer was used and the two most similar measurements averaged. Because coral plugs differed in their height relative to the scale bar, we applied plug-specific correction factors to reduce growth estimate biases related to plug height. This provided an accurate and precise estimate of coral growth.

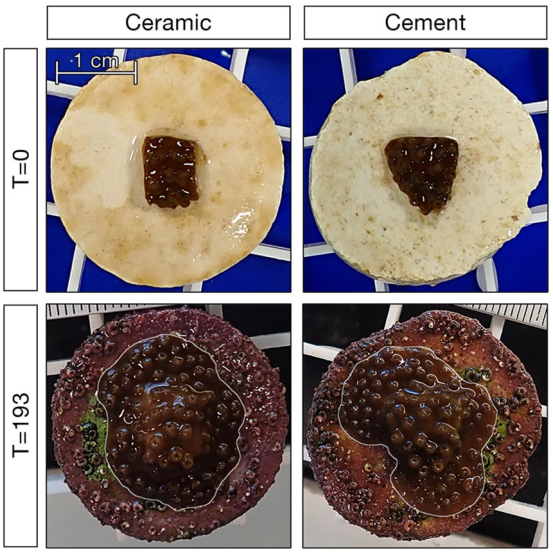

FIGURE 1 | Example of corals on ceramic and cement plugs at the start and end of the 193 day experiment. Corals in the bottom panels are outlined in white to differentiate them from fouling organisms on the plug.

We assessed the effects of genet and substrate type and their interaction on coral growth using a linear mixed effects model, model selection, and model weighting using R 3.6.1 (R Core Team, 2019) and RStudio 1.2.1578 (RStudio Team, 2015). Linear models were used as the vast majority of corals exhibited roughly linear growth rates over time (**Supplementary Figure 2**). Models were run using the *nlme* and *MuMIn* packages (Bartoń, 2020; Pinheiro et al., 2020) following Anderson (2007) and Zuur et al. (2009). The data were right skewed, so we applied a log10 transformation to approximate normality of residuals. General observation during the experiment suggested that ramet (i.e., parent colony) may affect growth. Because ramet was a potentially important source of variability that might otherwise obscure real effects of genet, substrate, or their interaction, we evaluated whether adding ramet as a random effect would improve the model. Four models were compared via AIC until we arrived at the optimal random effects structure: no random effects, ramet only, an interaction of ramet with substrate, and an interaction of ramet with genet (**Supplementary Table 2**). The optimal model included ramet alone as the random intercept, so it was added to all models that evaluated the fixed effects. To assess the significance of fixed effects, we compared the full model which included substrate, genet, and their interaction to three smaller nested models- one including genet only, one including substrate only, and one including substrate and genet but not their interaction. To complement the binary acceptance/rejection of model parameters and to minimize the problematic use of artificial thresholds (i.e., $p = 0.05$) to assess significance (Halsey, 2019; Hurlbert et al., 2019), we applied the *dredge* function from the *MuMIn* package (Bartoń, 2020) to determine the relative

importance of genet, substrate, and their interaction on coral growth rates. Finally, we calculated summary statistics including daily rate of growth and area doubling time for each group retained in the final models.

RESULTS

All corals in this experiment survived and experienced positive growth over the course of the experiment. On average, corals grew from a starting size of 0.57–3.08 cm^2, an increase in size of 545% (**Table 1**).

As a whole, colony growth was right skewed with a mean of 0.0125 ± σ 0.0062 cm^2/day (0.08774 ± σ 0.0434 cm^2/week) and a median of 0.0109 cm^2/day (0.0760 cm^2/week) (**Supplementary Figure 3**). There was significant variation among microfragments, with the fastest growing microfragments exhibiting an order of magnitude faster growth than the slowest microfragment (daily growth of 0.0031 vs 0.0348 cm^2/day or 0.0217 vs 0.2437 $cm^{2/}$week). Growth was also more variable for faster growing genets (**Table 1** and **Figure 2**).

The addition of parent colony (i.e., ramet) as a random intercept significantly improved model fit over a model with no random effects, indicating parent colony had a significant influence on coral growth (Likelyhood Ratio Test, L = 66.46, df = 1, $p < 0.0001$). This model was also more parsimonious than models which included a ramet effect that varied by genet or substrate (**Supplementary Table 1**). Similarly, model selection indicated that the optimal model included both genet and substrate as fixed effects. This model outperformed both the genet only model (LRT, L = 6.48, df = 1, $p = 0.0055$) and the substrate only model (LRT, L = 12.72, df = 3, $p = 0.0026$). However, adding an interaction between substrate and genet did not significantly improve model fit (LRT, L = 3.25, df = 3, $p = 0.1773$). Multi-model averaging indicated that out of all possible model configurations, 96.6% of model weights included genet as a factor and 91.2% included substrate as a factor, but only 14.4% of model weights included their interaction (**Table 2**).

Corals placed on in-house made cement plugs grew at 111.9% the rate of corals placed on mass-produced ceramic plugs (0.0132 vs 0.0118 cm^2/day, **Table 1** and **Figure 2**). There were also significant differences in coral growth rates between genets (**Table 1** and **Figure 2**), with corals from genet 14-3 growing at 216% the rate of corals belonging to genet XA.

DISCUSSION

Numerous factors can impact the outcomes of coral restoration including coral growth, success/survivorship, and cost-efficiency of coral propagation. Many of these factors are controllable by restoration practitioners, and thus represent valuable opportunities to optimize the restoration process. Here, we isolated two such factors, plug substrate and genet, to determine their effects on growth rates of *Acropora palmata* microfragments. To our surprise, we found that there was a slight, but significant increase in coral growth on cement plugs compared with commercially available ceramic plugs. Additionally, our cost calculations have determined that the ceramic plugs cost more money than purchasing the materials to produce cement plugs in house. Because of this difference, as well as the availability of the cement supplies at various retailers, the use of cement plugs produces an increase not only in coral growth, but also in cost-efficiency and accessibility. This becomes even more significant when considering that nascent coral restoration operations, including those in remote areas or with limited funding or resources, can use widely available materials to produce cost-efficient coral substrates.

Despite the growth and cost advantages of cement plugs, ceramic plugs still hold several distinct advantages. First, ceramic plugs are smoother and more regular in construction. This not only makes it easier to maintain labels on corals, but also

TABLE 1 | Summaries of growth rates, initial fragment sizes, and estimated doubling times (assuming linear growth from time = 0) of coral groups.

Genet	Substrate	Mean size (cm^2) at T = 0	Mean size (cm^2) at T = 193	Mean percent growth	SD percent growth	Mean growth rate (cm^2/day)	SD growth rate (cm^2/day)	Time to double initial size (days)
14-3	Ceramic	0.58	3.70	646	272	0.0161	0.0080	36
14-3	Cement	0.62	4.11	689	298	0.0171	0.0079	36
14-3	All	0.60	3.91	667	284	0.0166	0.0079	36
14-4	Ceramic	0.55	2.85	514	125	0.0123	0.0057	45
14-4	Cement	0.60	3.39	566	140	0.0137	0.0049	44
14-4	All	0.58	3.12	540	135	0.0130	0.0053	45
14-5	Ceramic	0.55	2.75	498	107	0.0112	0.0041	49
14-5	Cement	0.61	3.49	581	161	0.0136	0.0044	45
14-5	All	0.58	3.15	543	144	0.0125	0.0044	46
XA	Ceramic	0.49	2.00	415	92	0.0075	0.0019	66
XA	Cement	0.52	2.20	426	82	0.0080	0.0024	64
XA	All	0.50	2.09	420	87	0.0077	0.0022	65
All	Ceramic	0.54	2.83	520	185	0.0118	0.0062	46
All	Cement	0.59	3.33	570	208	0.0132	0.0062	45
All	All	0.57	3.08	545	198	0.0125	0.0062	45

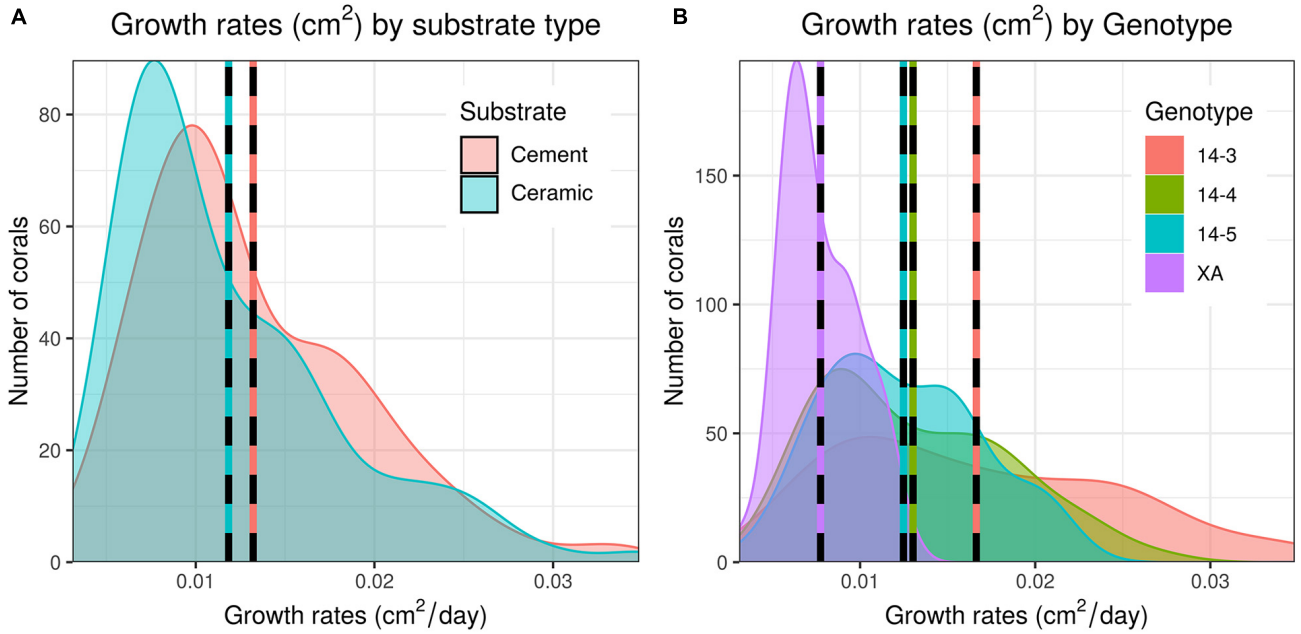

FIGURE 2 | Distribution of daily growth rates of corals between both substrate types **(A)** and genets **(B)**. Dashed lines indicate mean growth rates within each group. Untransformed data are shown here; data were Log10 transformed for statistical analysis.

makes it easier to hold, clean, and outplant them, as their stems are uniform in length, width, and angle. Ceramic plugs also provide a more uniform growth surface which may reduce variability during experiments. Furthermore, after cement plugs are constructed, they must be soaked for approximately six weeks before they are chemically inert and ready for use, a step that is not necessary when using ceramic plugs. Indeed, corals mounted on cement plugs initially grew slower than those mounted on ceramic, only overtaking ceramic plugs around 90 days into the experiment. While we did not explicitly test the reason for this, it is possible that leaching of cement plugs may have not been complete. This highlights the significant preparation time required when using cement plugs and is of particular importance for seasonal restoration efforts. Nevertheless, our results show that cement plugs made on-site can be a viable alternative to more expensive, ceramic plugs.

TABLE 2 | Results of multi-model averaging.

Model	Int	Sub	Geno	Sub*Geno	df	Log Lik
1	−1.835	+	+		7	105.536
2	−1.845	+	+	+	10	107.162
3	−1.858		+		6	102.294
4	−1.945	+			4	99.174
null	−1.969				3	96.008
Total Weight		91.20%	96.60%	14.40%		

*Parameters which are included in each model are designated by a (+) sign. Models are arranged by their relative weight. Int, Intercept; Sub, Substrate; Geno, Genet; Sub*Geno, Interaction effect between Substrate and Genotype; df, degrees of freedom; Log Lik, Log Likelihood.*

Coral growth significantly differed between substrates, but our results also show that substrate effects are dwarfed by the effect of genet. Faster growing genets can be fragmented more frequently, producing greater numbers of potential outplants (Baums et al., 2019). Identifying these fast-growing genets may help speed up the restoration process and success of restoration programs, maximizing output while minimizing cost. Additionally, because colony size is one of the main criteria for sexual maturity in corals, higher growth may allow corals to more quickly reach sexual maturity and increase fecundity at the colony level due to increased numbers of oocyte producing polyps (Álvarez-Noriega et al., 2016). Finally, rapid growth can allow outplants to better avoid size-specific mortality factors, such as corallivory or algae overgrowth (Drury et al., 2017).

Though rapid growth is a desirable coral trait, growth rate alone is not the only important consideration when choosing coral genets. Indeed, genet impacts variables other than growth (Williams et al., 2017; Pausch et al., 2018), and high growth rates may correlate with tradeoffs in other areas such as recovery from thermal stress (Ladd et al., 2017). Since the ultimate goal of most coral restoration is to create resilient, self-reproducing reefs, genets should also be screened for disturbance resilience as well as fecundity when possible. If individual genetic "stress testing" is not possible, coral gardening must be supplemented with restoration activities using coral larvae to increase genetic diversity (Lirman and Schopmeyer, 2016). With high genetic diversity, mass die offs might not be as common as some corals will be more thermally tolerant or disease resistant (Muller et al., 2018).

While the results of this study contribute to knowledge surrounding the efficient growth of *A. palmata* microfragments

ex situ, they are not necessarily indicative of how these microfragments perform as outplants in the wild. Indeed, since genet performance can interact with environmental variables, future work should consider the success and growth rates of microfragments once they have been reintroduced to their natural habitat, and individual practitioners should conduct growth experiments similar to the one described here not only to identify differences in growth rates within their own genet supply, but also to ensure the substrate effect we describe also holds under local environmental parameters. Importantly, if using growth experiments for restoration optimization purposes, it is critical to track growth rates over the same duration that corals are going to be raised *ex situ*, since growth rates can change over time and may interact with treatment group or season. Finally, there is a need to expand such work to other coral species used for restoration. As reefs continue to face increasing human stressors, coral propagation will continue to be a critical tool in maintaining reef survival and, eventually, restoration. Our study shows that decisions such as which substrate or genets to choose for restoration can have measurable and significant impacts on restoration speed, efficiency, and cost. As coral propagation expands, sharing such best practices will become increasingly more important for coral restoration to become more efficient and effective in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SH and RN conceptualized the study. EP and BW performed the experiments. RN analyzed the data. All authors wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.623963/full#supplementary-material

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METHODS
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3D Scanning as a Tool to Measure Growth Rates of Live Coral Microfragments Used for Coral Reef Restoration

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Rapid and widespread declines in coral health and abundance have driven increased investments in coral reef restoration interventions to jumpstart population recovery. Microfragmentation, an asexual propagation technique, is used to produce large numbers of corals for research and restoration. As part of resilience-based restoration, coral microfragments of different genotypes and species are exposed to various stressors to identify candidates for propagation. Growth rate is one of several important fitness-related traits commonly used in candidate selection, and being able to rapidly and accurately quantify growth rates of different genotypes is ideal for high-throughput stress tests. Additionally, it is crucial, as coral restoration becomes more commonplace, to establish practical guidelines and standardized methods of data collection that can be used across independent groups. Herein, we developed a streamlined workflow for growth rate quantification of live microfragmented corals using a structured-light 3D scanner to assess surface area (SA) measurements of live tissue over time. We then compared novel 3D and traditional 2D approaches to quantifying microfragment growth rates and assessed factors such as accuracy and speed. Compared to a more conventional 2D approach based on photography and ImageJ analysis, the 3D approach had comparable reliability, greater accuracy regarding absolute SA quantification, high repeatability, and low variability between scans. However, the 2D approach accurately measured growth and proved to be faster and cheaper, factors not trivial when attempting to upscale for restoration efforts. Nevertheless, the 3D approach has greater capacity for standardization across dissimilar studies, making it a better tool for restoration practitioners striving for consistent and comparable data across users, as well as for those conducting networked experiments, meta-analyses, and syntheses. Furthermore, 3D scanning has the capacity to provide more accurate surface area (SA) measurements for rugose, mounding, or complex colony shapes. This is the first protocol developed for using structured-light 3D scanning as a tool to measure growth rates of live microfragments. While each method has its advantages and disadvantages,

disadvantages to a 3D approach based on speed and cost may diminish with time as interest and usage increase. As a resource for coral restoration practitioners and researchers, we provide a detailed 3D scanning protocol herein and discuss its potential limitations, applications, and future directions.

Keywords: 3D scanner, microfragment, coral, coral restoration, growth rate, coral propagation, coral reefs, land nursery

INTRODUCTION

Coral reefs worldwide have suffered severe declines in cover and health as a result of negative anthropogenic impacts. In response, there have been increased investments in coral reef restoration activities to rehabilitate degraded populations and restore essential ecosystem services and functions (Boström-Einarsson et al., 2020). Recent developments in coral propagation techniques (e.g., microfragmentation; Forsman et al., 2015; Page et al., 2018) and other technological advancements have thus stemmed from the need for science-based interventions that are broadly applicable and can support upscaled restoration efforts (National Academies of Sciences, Engineering, and Medicine, 2019).

Microfragmentation, an asexual coral propagation technique with roots in the aquarium industry, has over recent decades been adopted and modified by coral restoration scientists to produce large numbers of corals for outplanting onto degraded reefs (Forsman et al., 2015; Page et al., 2018), thereby rapidly increasing live coral cover. Similar to *in situ* coral gardening techniques where coral fragments of branching species are mass-produced in underwater nurseries, microfragmentation takes advantage of the corals' ability to reproduce asexually and is not limited to branching species. Colonies are cut into small replicate pieces ("microfragments"), with optimal fragment size dictated by species and polyp size. The fragments are grown in a land- or field-based nursery and eventually outplanted onto a degraded reef or dead coral head, typically in arrays of replicate fragments that fuse to form a large colony quickly (Forsman et al., 2015; Page et al., 2018). Microfragmentation can be applied to any scleractinian coral species, but is especially effective for slow-growing massive or mounding species (e.g., brain and boulder corals) with adult colonies that do not tend to naturally fragment. This strategy has also shown to reduce the time to the onset of sexual maturity and first reproduction by producing puberty-sized colonies in a matter of years instead of decades (Koch et al., 2021). This technique is now being widely used by coral reef restoration practitioners for coral propagation (Boström-Einarsson et al., 2020).

For maximizing the adaptive potential of restored coral populations, it is recommended to propagate coral genotypes with one or more phenotypic traits predicted to be valuable in the future, such as low partial mortality, high wound healing rate, high fecundity, high bleaching resilience, disease resistance, and high growth rate (Hall and Hughes, 1996; Palmer et al., 2011a,b; Lirman et al., 2014; Kuffner et al., 2017; Muller et al., 2018; Baums et al., 2019). Tracking key traits in nursery populations will help restoration practitioners optimize nursery stocks and ensure that a diverse suite of potentially important traits are included in outplanting designs (Baums et al., 2019). For optimally managing coral nurseries, it is also recommended to establish consistent practical guidelines for collecting data (Baums et al., 2019; Boström-Einarsson et al., 2020). To address this point, and to add to the suite of available coral restoration tools, we developed a 3D scanning protocol for quantifying growth rates of live coral microfragments used in coral reef research and restoration projects. This precise and non-destructive method provides surface area (SA) measurements of live coral tissue in a high-throughput manner that is both accurate and reproducible. From these SA measurements, growth rates of living corals can be quantified over time, making 3D scanning a beneficial benchtop tool for obtaining standardized phenotypic quantifications in a laboratory setting.

3D scanning is a non-contact, non-destructive technology that uses light projection, a stereo-camera setup, a movable tray, and principles of photogrammetry to capture the shape and size of physical objects and produce a full digital 3D model of them. This model can be used for scientific measurements, such as SA. There are different types of 3D scanning technologies, with the main difference being the source of light. Laser scanning (e.g., light detection and ranging, LIDAR) utilizes optical amplification of coherent light to create points between the laser and the object being scanned, and is highly accurate (Veal et al., 2010b). Digital photogrammetry involves two-dimensional imaging at different angles, followed by triangulation via software to stitch the 2D images together into a 3D structure (Bythell et al., 2001). Another method is infrared or structured-light 3D scanning, which uses projected light and a camera system to emit light at the surface of the object (Veal et al., 2010b). Distortions in the projected light are used to create the object's surface geometry.

A variety of 3D approaches have been developed for capturing various scleractinian coral characteristics, including morphology, size, and growth (**Table 1**). For example, 3D laser scanning has previously been used to obtain SA measurements (Raz-Bahat et al., 2009) and morphological differences (Zawada et al., 2019) of various coral fragments. However, coral skeletons were used in these studies, rendering these protocols applicable to dead coral material only. The need for non-destructive assessments of living corals in a laboratory setting has led others to develop similar protocols including X-ray computed tomography (CT) and 3D modeling (Laforsch et al., 2008), but these techniques have drawbacks, including high instrument cost and long out-of-water exposure times. Others have measured live corals using structured-light 3D scanning to assess live corals that are larger and more complex than microfragments, but noted that

TABLE 1 | A non-exhaustive list of methods to measure surface area of coral reef sessile epibenthic organisms (e.g., corals, sponges, hydrocorals, algae).

Category	Method	Advantages	Drawbacks	Potential uses	References
Analog	Aluminum foil	• Simple • Inexpensive • Accurate • Easily applied • Rapid	• Destructive • Only works for smaller, simpler coral colonies • Cannot be applied *in situ*	Zooxanthellae densities per surface area	Marsh, 1970
	Wax-dipping				Glynn and D'Croz, 1990 Stimson and Kinzie, 1991 Chancerelle, 2000 Vytopil and Willis, 2001 Hoegh-Guldberg et al., 2005 Holmes et al., 2008 Naumann et al., 2009 Veal et al., 2010a
	Latex				Meyers and Schultz, 1985
	Dye-dipping				Hoegh-Guldberg, 1988
	Surface Index (SI) calculation	• Simple • Inexpensive • Non-destructive • *In situ*	• Not highly accurate	Field surveys	Dahl, 1973 Roberts and Ormond, 1987 Babcock, 1991 Alcala and Vogt, 1997 Bak and Meesters, 1998 Chancerelle, 2000 Fisher et al., 2007
Photogrammetry	Geometric calculation from 2D Imagery	• Non-destructive • Easily applied • Inexpensive	• Time-consuming	Laboratory research	Falkowski and Dubinsky, 1981 Rahav et al., 1991 Muscatine et al., 1989 Ben-Zion et al., 1991 Tanner, 1995 Holmes, 2008 Naumann et al., 2009 House et al., 2018 Mclachlan and Grottoli, 2021
	Stereophotogrammetry (3D reconstruction)	• Non-destructive • *In situ* Accurate (when using software and SfM) • Not restricted to one type of camera • Can capture texture and color	• Time-consuming for post-photo processing • Branching forms are difficult to do this with due to obscuring other branches. • Often requires specialized software expertise • 3D reconstruction softwares can be expensive.	Field surveys	Done, 1981 Fryer, 1983 Bythell et al., 2001 Cocito et al., 2003 Abdo et al., 2006 Courtney et al., 2007 Jones et al., 2008 Burns et al., 2015 Figueira et al., 2015 Lavy et al., 2015 Burns et al., 2016 Ferrari et al., 2016 Raoult et al., 2016 Ferrari et al., 2017 Pinheiro et al., 2020 Raoult et al., 2017 House et al., 2018 Lange and Perry, 2020 Million and Kenkel, 2020
	Laser scanning	• Non-destructive • High accuracy and precision • Rapid • With a digital camera add-on, can capture texture and color	• Often requires specialized software expertise • Scanner hardware is expensive	Laboratory research	Holmes, 2008 Raz-Bahat et al., 2009 Zawada et al., 2019
	Structured light scanning				Veal et al., 2010b Enochs et al., 2014 Reichert et al., 2016 This paper

(Continued)

TABLE 1 | Continued

Category	Method	Advantages	Drawbacks	Potential uses	References
	• X-Ray computed tomography (CT) scanning	• High-precision • Can capture complex structures	• Expensive • Inaccessible for routine laboratory work	Skeletal densities	Bessat et al., 1997 Kaandorp and Kubler, 2001 Kaandorp et al., 2003, 2005 Kruszyński et al., 2007 Laforsch et al., 2008 Naumann et al., 2009 House et al., 2018

reproducibility decreases with increasing complexity (Enochs et al., 2014; Reichert et al., 2016), a problem less likely to occur with microfragments.

Newer digital techniques have supplemented more traditional analog methods for measuring coral SA (e.g., aluminum foil wrapping, wax- or dye-dipping) which measure skeletal SA —not tissue SA specifically. These methods are inherently destructive, rendering them inapplicable to living corals or repeated measures of growth (**Table 1**). Other commonly used methods for measuring SA of live corals include photogrammetry or geometric calculations (**Table 1**). Buoyant weighing coral fragments is also a simple and non-destructive method for assessing coral growth (Franzisket, 1964; Bak, 1973, 1976; Jokiel et al., 1978; Dodge et al., 1984); it relies on using relatively inexpensive equipment to weigh a coral underwater and then predicting from this weight, the weight of the skeleton (Davies, 1989). Thus, this approach is different to those tested herein in that it measures skeletal accretion ("calcification") rates, not growth rates based on changes in tissue SA.

While the field of photogrammetry has been used in coral research for decades (e.g., Done, 1981; Fryer, 1983), improving technology has made one particular technique recently popular in biological studies: stereophotogrammetry. Stereophotogrammetry, sometimes referred to as Structure from Motion (SfM), uses software and a series of overlapping 2D images taken at different angles on a feature (like an object or landscape) to accurately construct a 3D model of that feature (Raoult et al., 2017). Though SfM is typically employed by moving a camera around a static feature (e.g., Raoult et al., 2017), it can also be employed by moving an object around a static camera or scanner. In coral conservation, SfM has emerged as a popular, non-invasive underwater tool that creates accurate 3D digital models of coral colonies for assessing various metrics — including growth—by reconstructing 3D volume and topology from overlapping 2D image sequences (Raoult et al., 2017). While this method has proven successful for *in situ* applications, use with microfragments in a land-based nursery setting has yet to be explored.

The protocol presented herein incorporates structured-light 3D scanning as a tool to measure growth rates of live microfragments but, similar to other studies, we find this method to be highly precise, reproducible, minimally invasive, and capable of rapidly processing large sample sizes. After developing the protocol, we applied it to a subset of corals and compared the outcomes of the 3D approach to those of a more traditional 2D imagery approach, based on accuracy, speed, and reproducibility.

Combining 3D scanning technology with coral microfragmentation to measure coral growth rates has several applications within coral reef restoration science. From an applied perspective, microfragmentation can be used to upscale production, and 3D scanning technology can be used to help select candidate genotypes to propagate. From a research perspective, microfragmentation can be used to produce biological replicates of different genotypes that are exposed to different stressors for identifying resilience or resistance, with 3D scanning used as a tool for assessing phenotypic responses such as growth. 3D scanning can also be used to assess growth rates of sexual recruits, which start out as microscopic individuals and grow in size and complexity over time. Apart from microfragmentation and biological replication, population-level responses may be obtained for experiments involving different treatments and cohorts of sexual recruits. Finally, 3D scanning technology can be used for detecting phenotypic differences other than SA, such as polyp size and shape or rugosity.

MATERIALS AND EQUIPMENT

Corals and Microfragmentation

Sexual recruits of the elkhorn coral, *Acropora palmata*, were raised in captivity at Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research and Restoration (Summerland Key, FL, United States) for approximately 4 years prior to the start of the study in 2019. Over that time, the corals were repeatedly propagated asexually via microfragmentation for the purpose of outplanting and restoration. To microfragment corals for this study, we cut stock corals ($\sim$7 cm^2) into $\sim$0.5 cm^2 microfragments (0.48 $\pm$ 0.10 SD cm^2) using a wet C40 diamond band saw (Gryphon Corporation, Sylmar, CA, United States) (Page et al., 2018). Using extra thick cyanoacrylate super glue gel (Bulk Reef Supply, Golden Valley, MN, United States), we secured the microfragments to $\sim$3 cm^2 circular mounts ("plugs"). We maintained the corals in a common garden setting in flow-through fiberglass raceways with ambient seawater conditions ($\sim$26–28°C, $\sim$7.7–8.0 pH, and $\sim$38 ppt salinity), which we monitored twice daily using a YSI Professional Plus handheld multiparameter meter (Xylem Inc., Yellow Springs, OH, United States).

3D Approach

The HDI Compact C210, a 3D desktop light-structured scanner manufactured by Polyga (Burnaby, BC Canada), is a durable scanner suitable for working in wet laboratory environments. The capturing unit uses LED structured-light technology and contains a pair of two megapixel cameras with an accuracy of up to 35 μm. The light source projects a series of patterns onto the target and the resulting distortions become incorporated into the 3D digital model. The device has a field of view of 71 × 100 × 154 mm, which is sufficient to effectively capture scans of corals ranging from small microfragments to larger fragments with varying morphology. The camera rig is paired with a rotary turntable to automate and quicken the 3D scanning process. As the target turns, multiple scans are captured which are then aligned and merged to create a complete digital 3D model. This requires the use of Polyga's proprietary software, FlexScan3D, which also provides automated post-processing capabilities to streamline the 3D scanning process including cleaning, alignment, merging, and hole-filling. Importantly, the FlexScan3D software is general-use and can be used with any kind of 3D scanner. For general user guidelines, and system and software setup, refer to the FlexScan3D User Manual v3.3.5.8 (LMI Technologies 2015).

One limitation of SfM photogrammetry generally (including 3D scanning) is the need for distinct reference points on the object of interest. Often, coral microfragments (which are often fairly uniform in feature) are mounted on circular disks, making it difficult for software to determine relative position of subsequent scans without assistance. To standardize fragment orientation and reduce alignment time, we created a coral stand that has unique geometry on four sides (see **Supplementary Figure 2** and the .obj file in Supplementary Materials for a printable file). This greatly assisted in both automated and manual image assembly.

2D Approach

While any digital camera will suffice, we used an Olympus Tough TG-4 waterproof camera to accommodate working around seawater. Our setup consisted of a piece of egg crate light panel (styrene lighting diffuser) used to stabilize the plugs during image capture, a ruler for size reference, and a whiteboard to display the unique coral ID. We analyzed images for surface area (SA) using ImageJ software, an open-source, Java-based application (Schneider et al., 2012).

METHODS

Objectives

The objectives of this study were to develop a standardized protocol for generating accurate and precise 3D models (hereafter, "meshes") of coral microfragments to obtain surface area (SA) measurements of live tissue, and to determine whether this approach could be used to accurately quantify microfragment growth rates by comparing the 3D approach to a 2D methodology based on digital photography and ImageJ analysis and then assessing accuracy and speed.

The following generalized protocol is meant to guide users regardless of the model of structured-light 3D scanner used. For settings specific to the HDI Compact C210 3D scanner and its FlexScan3D software, refer to Supplementary Materials and **Supplementary Figure 1**. Recently, Polyga released an option to purchase the FlexScan3D software as a standalone product, rather than purchasing in tandem with the HDI Compact C210 3D scanner[1]. This ultimately enhances accessibility and applicability of our protocol as the proprietary hardware is no longer necessary.

Procedure

General Workflow: For obtaining SA measurements of live microfragments to measure coral growth rates over time using a structured-light 3D scanner, the protocol has two main phases, starting with scanning of all samples, followed by post-processing of the 3D models (**Figure 1A**). The workflow outline is as follows: load software, calibrate scanner, adjust scanner settings, prepare coral sample by drying and inducing tentacle retraction, place coral on stand, adjust exposure settings, scan, return coral. Once all of the corals are scanned for a particular time point, processing of the 3D models begins with alignment and merging of the replicate scans. Then the 3D model ("mesh") is constructed and all non-coral elements are removed from the model, including the stand and coral mount. If necessary, holes (i.e., gaps in coverage) are manually filled and finally, SA measurements (mm^2) are extracted. The entire process, for one coral, can be completed in under 14 min with an air-exposure time of less than 3 min. Alternatively, a traditional 2D approach based on photography consists of setup, image capture via digital photography, scaling, tracing and extracting SA measurements using ImageJ (**Figure 1B**).

Image Acquisition

For this study, we fragmented *Acropora palmata* ramets from four genotypes into 16 microfragments, with four replicates per genotype. We then mounted the fragments on ceramic or cement plugs. After a short-term recovery period in *ex situ* aquaria to ensure health of microfragments, we prepared corals for measurement. Before measuring we agitated corals in the seawater holding bin to induce tentacle retraction, as tentacles can introduce error into SA measurements. We then gently dried each plug with a soft towel to absorb extra water before being mounted on the custom stand and placed in the scanner's camera frame. From here, we used the scanner's preview feature to adjust exposure for optimal scan quality. Dual exposure was necessary to pick up contrasting reflective surfaces, so we used the High Dynamic Range (HDR) setting. We used the rotary table to capture 12 scans (one at every 30°) of each coral plug mounted to the stand (**Figure 1A**) and followed standard operating procedure as outlined in the FlexScan3D User Manual to calibrate and load the scanner and its software.

Aligning, Merging, and Finalizing Scans

After individual scans of the coral from multiple angles have been acquired, they must be aligned. In the software used herein,

[1]https://www.polyga.com/flexscan3d-software/

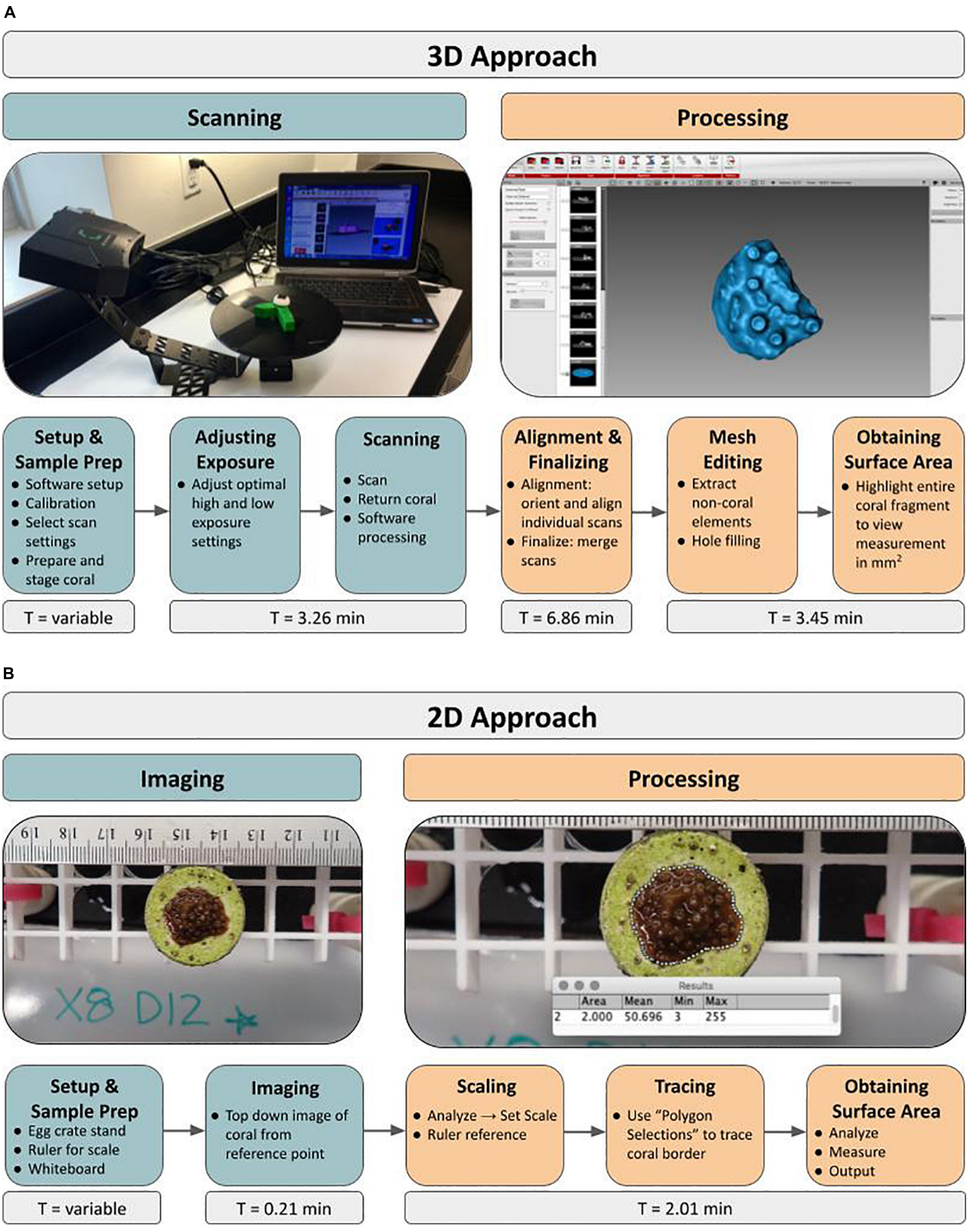

FIGURE 1 | Novel 3D and traditional 2D approaches to quantifying growth rates of live coral microfragments using surface area measurements. Workflow depicts major steps involved in both the 3D scanning approach **(A)** as well as the 2D photography approach **(B)**. The initial Setup and Sample Prep time ranges from 0.29 to 0.63 min in the 2D approach and from 4.75 to 6.20 min in the 3D approach.

we aligned our scans using the mesh geometry of the scans. Use of the custom stand with distinct asymmetrical geometry resulted in more successful alignment during this step when compared to alignment of scans that did not include the stand. After alignment, the software is able to merge scans into one object, with two options, Smooth Merge or Precise Merge. We found that the Smooth Merge function created complete meshes, while Precise Merge led to more gaps in the scanned coral. While assumptions are made in the Smooth Merge function to smooth over the gaps of individual scans, the function of the software is standardized and consistent. Conversely, if Precise Merge was used, the user of the scanner would need to fill in the gaps of the mesh manually using the Hole-filling tool. This creates the possibility for introducing human error and making assumptions that either over- or underestimate the actual SA. After a complete, continuous 3D mesh has been generated, the last step is to finalize the mesh into one 3D object, which can no longer be separated into individual scans.

Mesh Editing

Some scan-editing softwares have capabilities for post-processing of finalized 3D mesh objects. One tool worth mentioning is Hole-filling. While the Hole-filling tool was not required for the *A. palmata* fragments herein, this tool may be needed when scanning other, more rugose coral species to ensure complete 3D meshes. Because Hole-filling has the potential to introduce assumptions to the meshes, we recommended focusing on construction of a complete mesh via scans as opposed to relying on hole filling. Hole-filling can be avoided by properly adjusting exposure prior to scanning.

Obtaining Surface Area

To obtain an accurate SA, we edited the finalized 3D meshes to remove all non-coral structures, such as the stand and the plug. We did this by manually highlighting sub-selections of the mesh with the cursor, and then deleting these sub-selections until only the coral microfragment remained. When completing this step, it is important to distinguish the coral tissue border from the plug and other fouling organisms. For certain corals or on heavily fouled plugs, it may become necessary to reference a photograph of the coral to determine the true tissue boundary.

After editing the 3D mesh to solely encapsulate the microfragment, we measured SA by highlighting the entire remaining coral fragment and measuring the SA in mm^2 within the software. This value was manually exported to a spreadsheet for data collection and tracking.

Method Validation and Comparison to 2D Approach

Image Acquisition

To compare growth rate estimates generated by 3D scanning methodology, we took bi-weekly 2D images of each coral microfragment ($n = 16$) throughout the 213-day experiment. Images were captured using a digital camera and subsequently analyzed in ImageJ (Schneider et al., 2012). Setup included a piece of egg crate used to hold the coral plugs, a ruler for scale, and a whiteboard to display the unique coral ID. We briefly removed corals from their tanks to be photographed, resulting in only a few minutes of air exposure. We took each image directly above the coral (∼30 cm height) with the scale bar included in the frame (**Figure 1B**). To account for variations in plug height and distance to camera, we applied correction factors to 2D images using additional calibration photos taken at three time points.

Processing

To obtain the SA of a coral fragment in ImageJ, we first set the scale in ImageJ using the scale bar tool and the ruler in the image. We then traced the outline of the living microfragment tissue using the Polygon Selections tool with two separate observers to get an idea of precision and to ensure agreement and accuracy. These traces were averaged to get a mean SA, which we obtained directly from the scanner software in cm^2. In rare cases when SA measurements were not within 5% agreement, a third observer independently traced the outline of the coral microfragment and was used to calculate a new SA estimate using the two most similar measurements.

Pause Points

The efficiency of the 3D scanning process depends on the processing power and speed of the computer used, as well as the number of corals scanned at any one time. A common pause point occurred after a set of coral microfragments were scanned and then moved from their holding bin back to their tanks to maintain their health. Then, a new subset was brought from their tanks to the scanner. Additionally, to support high-throughput processing, which incorporates a pause point between scanning and data extraction, all corals can be scanned at once followed by mesh generation and measurement acquisition at a later time.

Statistical Analyses

We performed all analyses in R v.3.6.1 (R Core Team, 2019). To determine the effect of scanning methodology on coral SA measurements at any one time, we constructed a mixed effects model using the *lme* function in the *nlme* package (Pinheiro et al., 2020). Coral area was the dependent variable; time, data type (photo or scan), and their interaction were independent variables. To account for repeated measures of a coral across time, we included coral ID as a random effect.

To determine the effect of scanning methodology on coral growth rates, we used the 2D image capture events ($n = 13$ each) and 3D scan events ($n = 8$ each) to calculate daily coral growth rates for individual corals over the course of the 213-day experiment. Daily growth rates were determined by fitting a linear regression to the SA data since coral growth was generally linear over time (Papke et al., 2021) with daily coral growth as the dependent variable and data type (photo or scan) as the independent variable using the *gls* function in the *nlme* package (Pinheiro et al., 2020). Because slopes inherently encapsulate repeated measures, no autoregression factor was applied.

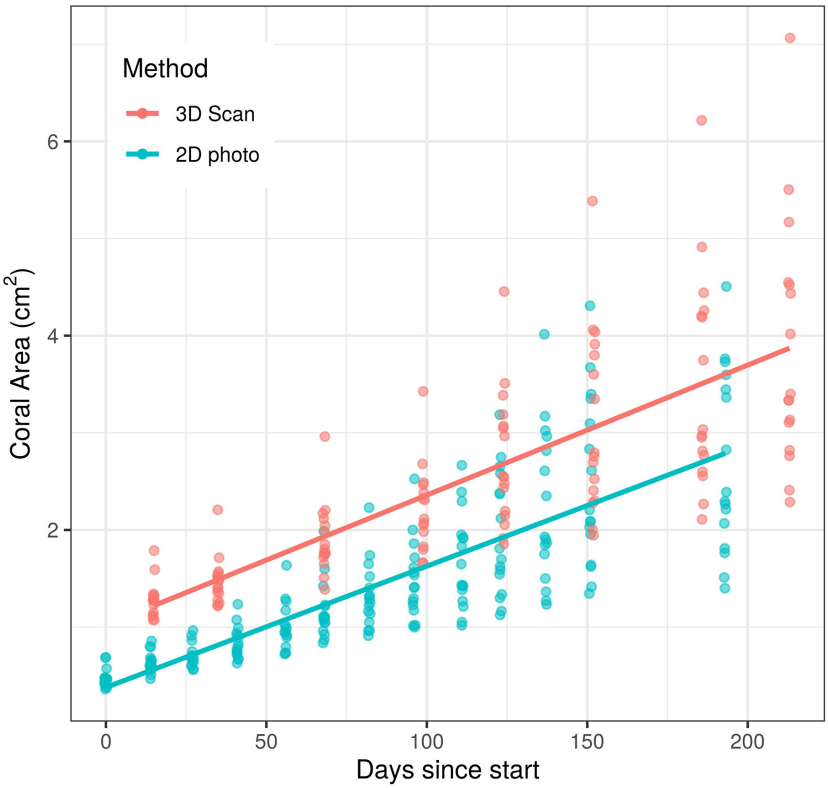

FIGURE 2 | Effects of time and measurement method on coral area measurements over a *c.a.* 200 day growth experiment. There was a significant effect of both time ($p < 0.0001$) and measurement method ($p < 0.0001$) on surface area measured, but the effect of measurement method did not change over time ($p = 0.208$).

RESULTS

Method Validation–3D vs. 2D Comparison

Unsurprisingly, both time and measurement type had significant effects on coral area ($p < 0.0001$ for each effect). However, the time * measurement type interaction had no significant effect on coral area ($p = 0.208$, **Figure 2**), indicating that the significant effect of measurement type on coral area is consistent across time points. Similarly, the analysis of individual coral slopes indicated that measurement type has no effect on estimates of coral growth ($p = 0.158$, **Figure 3**). When comparing initial and final SA measurements between the two approaches, results show that the scanner consistently estimates higher values than the photos (**Figure 3**). This is expected as 3D measurements can better account for variation in SA due to morphological complexities, which may go undetected by a 2D photo approach, but are more biologically relevant than just area covered anyhow.

Reproducibility

In general, results were highly reproducible for both methods. For the 2D photography approach, the mean deviation between surface area measured by two observers was 0.0151 cm^2. Only 2.7% of measured corals exceeded the 5% agreement threshold after the first two measurements, and thus required a third observation. For those cases in which the first two observations were not within 5% agreement, the mean deviation was 0.0873 cm^2. Variability between multiple SA measurements obtained from repeated 3D scans of the same coral at the same time point was negligible (see section "Mesh Editing").

Timing

For trained observers, estimation of microfragment SA was significantly faster for 2D photography (2.01 ± 0.26 min) than 3D scanning (14.76 ± 2.97 min), even after taking into account the need for two observers (Mann Whitney *U*-test, W = 2,250, $p < 0.0001$, **Table 2**). Furthermore, timelines associated with the 3D methodology are further impacted by the processing time of the FlexScan3D software, which can vary (e.g., 15 s–5 min) depending on the processing power of the computer, as well as file storage capabilities or devices (e.g., external hard drive vs. a flash drive). Faster computers will significantly reduce processing time from what is described here.

Coral Health

For both methods, corals remained healthy throughout the experiment. Indeed, we witnessed no mortality or unusual levels of stress among the experimental corals, indicating 3D scanning poses little threat to coral health when applied properly. During coral placement, exposure adjustment and scanning stages of the 3D approach, corals are exposed to air for approximately 3

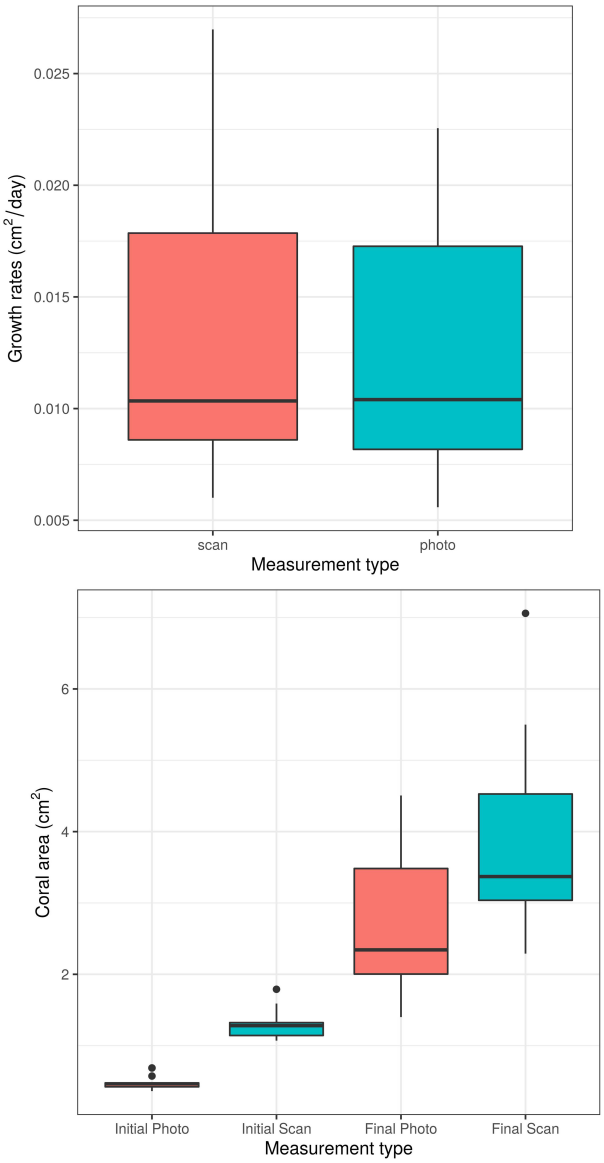

FIGURE 3 | Boxplots of daily coral growth rates **(top)** and of initial and final coral area **(bottom)** as measured by photo and 3D scanner. The two measurement methods do not significantly differ in their ability to measure coral growth ($p = 0.158$).

TABLE 2 | Average time required for an experienced operator to complete each stage of coral scanning and processing or photography.

	3D scanning ($n = 75$)		2D photography ($n = 30$)	
Stage	**Duration (min)**	**SD (min)**	**Duration (min)**	**SD (min)**
Image acquisition	3.26	0.53	0.21	0.03
Export	1.19	0.17	–	–
Post-Scan	6.86	2.57	–	–
Cropping	3.45	1.71	2.01	0.26
Total	14.76	2.97	2.01	0.26

For the scanner, Image Acquisition describes time from when the coral was placed on the stand to when the software finished processing scans- corals were only exposed to air during collection of scans (first 1–2 min of scanning). For photography, Image Acquisition includes preparation of the photography surface. For 3D scanning, Export describes time needed to export unaligned scans for backup while Post-Scan describes time needed to align scans manually and generate a final mesh. Cropping refers to the time needed to remove the base and plug from the scan of the coral tissue, obtain a surface area measurement, and export the final aligned mesh for backup. Cropping for photography refers to the time needed for two subsequent observers to import a photo, calibrate the scale, outline the coral, and get an estimate of surface area. Duration of scanning and export depends largely on machine capabilities, while the duration of post-scan and cutting stages depends largely on technician speed. For both methods, transfer of data (photo, scans) from one device to another is not considered.

min. As corals are routinely transported in air for short periods of time during restoration and outplanting activities, 3 min is not expected to harm or stress the corals, as no signs of stress were observed in this study. Additionally, aerial and bright light exposure were observed to have no effect on coral health, evidenced by the extension of their tentacles shortly after being returned to their tanks.

DISCUSSION

Three-dimensional measurement techniques are not new to coral reef research. Several 3D methodologies have been used in previous studies to measure surface area of corals, and landscape-scale studies using photogrammetry with simple computer programs have been around for 40 years (e.g., Done, 1981; Fryer, 1983; see **Table 1**). However, there has been little evaluation of the utility of 3D scanning as a tool for measuring coral microfragment area or growth. The present study demonstrates that 3D scanning is a reliable method to obtain accurate measurements of absolute SA of coral microfragments when compared to 2D photography, and that 2D photography underestimates absolute SA of microfragments. Indeed, 3D estimates of coral area were significantly and consistently higher than estimates created from a common photographic method (**Figures 2**, **3**), as observed previously (House et al., 2018). This is likely because 3D scanners (e.g., HDI Compact C210) are able to quantify not only total convex SA in the 2D plane, but also the rugosity of the coral polyps themselves (Enochs et al., 2014). Differences in SA estimates generated by 3D technologies vs. other methods have been observed in previous studies. For example, Bythell et al. (2001) demonstrated that foil-wrapping techniques overestimated coral SA by 20% when compared to estimates by *in situ* 3D photogrammetry. In a study comparing 3D laser scanning and four traditional methods (wax coating, foil wrapping, methylene blue dipping, and caliper measurements), the 3D laser scanner produced more accurate coral SA estimates (Raz-Bahat et al., 2009).

In the present study, even though 3D estimates of coral SA were more accurate, the difference in area estimates between the 2D and 3D scanners were constant over time (and coral size), indicating that the difference in area estimation between the two methods seems to be constant when testing microfragments. House et al. (2018) also reported similarities in the scaling relationship between 3D and 2D SA estimates of corals. In our study, estimates of microfragment growth rates were almost identical between the two methods, indicating that 2D

photography produces an accurate estimate of absolute growth rate, if not absolute coral SA, as long as corals maintain a relatively flat morphology. For larger, more rugose corals, use of a 3D scanner may be more critical (Reichert et al., 2016). Similarly, for coral species with large polyps (such as *Montastraea cavernosa*), deviations between SA estimates may be more apparent between methods, underscoring the importance of high resolution 3D scanning.

Though both the 2D and 3D approaches produce similar estimates of growth rates, 2D methods have several advantages. First, the 2D photography method is intuitive and simple to learn, and requires little specific training outside of ImageJ. Furthermore, 2D methods are more time efficient- to image, analyze, and obtain a SA measurement from a coral takes approximately 7.3 times longer with the 3D approach than the 2D approach (**Table 2**). In the same amount of time, a much larger number of corals can be measured with 2D methods than 3D methods. However, there are exceptions. For example, handheld 3D scanners have been used to scan small corals (e.g., 10 cm height) in approximately 60 s (Reichert et al., 2016). In the present study, a recommended next step would be to investigate ways to reduce the amount of processing time currently required of users implementing the 3D approach. One possible solution is to explore the feasibility of measuring corals underwater. Developing a method whereby users can measure corals without first removing them from water would reduce time necessary to prepare the corals (e.g., transferring coral samples from water to the stand) prior to scanning.

The main advantages of the 3D approach to assessing growth are accuracy, and to a lesser degree, precision. While the 2D approach was precise (see results), it still uses human observers to measure coral SA. In contrast, repeat scans and measurements were not necessary with the 3D scanner, as the software was able to generate complete 3D meshes that were visually identical to the live corals. Both methods have a subjective point in analysis where living coral tissue must be differentiated from dead tissue or other features like glue. Careful post-processing of the 3D scans in the cropping phase is required to ensure that only live coral tissue remains in the scans (Enochs et al., 2014). Here the 3D method offers another advantage in accuracy. Unlike the 2D method, where days or weeks may pass between when a photo is taken and when it is analyzed, an operator that creates meshes as they scan each coral can immediately identify a "problem area" on the scan where it is hard to differentiate living coral from other material and can then reference the live coral in real time to distinguish living tissue from other features. In this experiment, the live tissue borders were easily distinguished from the plug and differences in cropping accuracy between technicians were negligible.

In addition to advantages in precision, 3D scanning gathers a more accurate estimate of coral SA when compared to a 2D photography method (**Figures 2**, **3**). As corals become more rugose (i.e., begin mounding or branching), this disparity increases (Bythell et al., 2001; Reichert et al., 2016). Similarly, 3D scanning can be particularly useful to measure the SA of corals that are not on uniformly flat surfaces, such as young colonies that may have settled on experimental settlement surfaces in the field. Furthermore, high-resolution 3D scans can be used to extract more data about coral growth than a 2D image or analog methods (like foil) can, such as information about rugosity. Finally, and most importantly, 3D scanning provides a more accurate estimate of *absolute* coral SA, even when corals are small. In the present study, 3D scanning data consistently estimated a significantly higher SA than 2D photography. For experiments measuring the effects of local variables (e.g., growth substrate) on coral size, such as 2D methods, are likely sufficient. However, to make results comparable across many studies, accurate measurement of coral SA is essential. For this reason, 3D scanning is an optimal method to gather coral SA data for meta-analysis. As coral restoration science continues to grow, the role of such meta-analyses will likewise increase. Thus, having standardized methodologies for obtaining important trait data, like growth rate, across independent groups can help to advance collaborative restoration research more effectively.

The digital techniques used to assess coral area have been compared to analog methods in other studies. One of the most common analog physiological metrics used for coral growth estimations is buoyant weighing. Buoyant weight is a non-destructive, standard metric used to measure coral skeletal growth (Jokiel et al., 1978). However, buoyant weighing of microfragments is neither feasible nor practical for their growth assessments. Microfragment or sexual recruit growth is much more fine-scale within the first years of a coral's development, and it is difficult to capture these slight changes using the standard buoyant weighing technique over a relatively short timescale without having to modify the methodology (Jokiel et al., 1978; Davies, 1989). Indeed, buoyant weighing techniques are recommended for longer-term assessments of coral skeletal growth (Schoepf et al., 2017). Therefore, 3D scanning may be a viable alternative to the buoyant weight technique when measuring growth of microfragments or sexual recruits.

Despite the clear advantages 3D scanning has under certain use cases, we acknowledge limitations inherent in 3D scanning, including maximum coral size, scan time, and cost. Costs associated with 3D scanning include the hardware (i.e., scanner, computer, data storage devices) and maintenance of the scanning software service contract. However, since FlexScan3D is a hardware agnostic, standalone software product, costs may be reduced by using alternative/cheaper scanners. For 2D photography, a digital camera is the greatest expense beyond user time, and our study suggests that 2D methods may be as reliable as 3D scanning when researchers are primarily interested in only growth of small microfragments on flat substrates. In this case, a small number of scans can be taken of a subset of focal corals to generate a SA correction factor between 2D imagery and 3D SA. This would be more efficient than scanning every coral at all time periods but would provide the advantage of allowing for true comparisons of coral area over many studies.

Structure-from-Motion (SfM) photogrammetry has been implemented as an efficient and cost-effective method for measuring coral SA and volume, when combined with morphotypic data (House et al., 2018). SfM technologies have become increasingly more common among coral reef researchers and restoration practitioners for *in situ* use, especially

given that open-source software programs are available (Figueira et al., 2015; Lavy et al., 2015; Gutiérrez-Heredia et al., 2016; House et al., 2018). Unsurprisingly, this has opened many areas for future research. For example, since the corals in this study remained healthy and showed no signs of tissue loss, it was unnecessary to distinguish between live and dead coral tissue material. A recommended next step would be to compare the 3D scans of healthy and stressed corals to determine if, for example, the scanner can distinguish between live tissue, bleached tissue and exposed skeleton if a coral is experiencing tissue recession. This information would provide further insight into the sensitivity and precision of the 3D approach, as well as its applicability to stress tests where different coral genotypes are screened for resilience to a variety of stressors. Similarly, it would be useful to evaluate how this method performs with more rugose species which can have significant three-dimensional structure even as microfragments (e.g., *Montastraea*). Such studies will further contextualize the role of 3D scanning in future coral research and restoration.

CONCLUSION

Here we show that 3D scanning provides comparable reliability to 2D ImageJ analysis within a single study and that 3D scanning is a viable way to measure surface area of coral microfragments. While 3D scanning may not yet be as time efficient as 2D photography, its advantages are numerous. 3D scanning provides a precise, accurate measurement of absolute SA which is directly comparable across studies, providing a standardized measurement useful for networked experiments, meta-analysis, and synthesis. 3D scanning is also preferable to 2D methods for rugose, mounding, or complex colony shapes. Since growth morphotype is variable across genotypes and substrates, we suggest at a minimum that a representative subsample of corals be measured with 3D scanning to facilitate cross-study comparisons. For short-term experiments with small microfragments that are likely to experience "sheeting" growth, 2D analysis may be sufficient for in-house experimental purposes when the primary variable of interest is growth, not area *per se*. Yet, it is increasingly important to standardize coral growth metrics across different research groups and coral restoration practitioners. The use of more standardizable methods, such as 3D scanning, represents a reliable way to meet this need.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Dryad (https://doi.org/10.5061/dryad.4b8gthtc1).

AUTHOR CONTRIBUTIONS

HK, AC, and RN conceptualized the study. AD developed the protocol. BW and RN optimized the protocol. BW conducted the study and collected all 3D and 2D measurements. RN analyzed the results. HK, BW, AD, AC, and RN wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.623645/full#supplementary-material

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Enhancing Coral Survival on Deployment Devices With Microrefugia

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Surviving after settlement through the first year of life is a recognised bottleneck in up-scaling reef coral restoration. Incorporating spatial refugia in settlement devices has the potential to alleviate some hazards experienced by young recruits, such as predation and accidental grazing, and can increase the likelihood of survival to size-escape thresholds. Yet optimising the design of microrefugia is challenging due to the complexity of physical and biological processes that occur at fine spatial scales around a recruit. Here, we investigated the effects of microhabitat features on the survival of *Acropora tenuis* spat in a year-long experimental field deployment of two types of artificial settlement devices—grooved-tiles and lattice-grids—onto three replicate racks on a shallow, central mid-shelf reef of the Great Barrier Reef. Spat survival across device types averaged between 2 and 39% and about half of all devices had at least one surviving coral after a year. While the larvae settled across all micro-habitats available on the devices, there was strong post-settlement selection for corals on the lower edges, lower surfaces, and in the grooves, with 100% mortality of recruits on upper surfaces, nearly all within the first 6 months of deployment. The device type that conferred the highest average survival (39%) was a tile with wide grooves (4 mm) cut all the way through, which significantly improved survival success over flat and comparatively featureless control tiles (13%). We hypothesise that the wide grooves provided protection from accidental grazing while also minimising sediment accumulation and allowing higher levels of light and water flow to reach the recruits than featureless control devices. We conclude that incorporating design features into deployment devices such as wide slits has the potential to substantially increase post-deployment survival success of restored corals.

Keywords: coral settlement, spat, post-settlement survival, microrefugia, grooves, outplant, deployment, coral restoration

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INTRODUCTION

Coral populations are declining globally (Gardner et al., 2003; Bruno and Selig, 2007; De'ath et al., 2012; Hughes et al., 2017), stimulating widespread efforts to mitigate further losses, enhance the recovery of existing populations, and potentially increase reef resilience through coral restoration programs (Boström-Einarsson et al., 2018, 2020; Bay et al., 2019). Seeding a recruitment-limited

reef with deployment devices carrying sexually derived and newly settled coral spat (Okamoto et al., 2008; Chamberland et al., 2015, 2017) is one of the interventions being tested and refined (Bay et al., 2019). The benefits of using sexually produced coral propagules in reef restoration include improvements in genetic diversity, scalability and cost (Baria-Rodriguez et al., 2019; Doropoulos et al., 2019; Gibbs et al., 2019; Randall et al., 2020), and if harnessing spawn slicks (Heyward et al., 1999, 2002; Doropoulos et al., 2019), retention of species diversity and community composition (Heyward et al., 1999; Doropoulos et al., 2019). Seeding reefs with already-settled coral spat also, at least temporarily, overcomes challenges associated with the settlement process, including a lack of available substrate or settlement cues (Kuffner et al., 2008; Webster et al., 2011, 2013), and the presence of settlement inhibitors (Kuffner et al., 2006; Arnold et al., 2010; Webster et al., 2015; Speare et al., 2019). Yet post-settlement mortality can be exceedingly high (>99%) in some habitats and under various environmental conditions (Babcock, 1985; Hunt and Scheibling, 1997; Wilson and Harrison, 2005; Vermeij and Sandin, 2008; Penin et al., 2010, 2011; Ritson-Williams et al., 2010; Trapon et al., 2013; Miller, 2014; Suzuki et al., 2018), potentially diminishing the benefits of the seeding technique.

High post-settlement mortality in corals can be caused by accidental grazing by fishes (Baria et al., 2010; Penin et al., 2010, 2011; Trapon et al., 2013; Gallagher and Doropoulos, 2017), competition with other benthic organisms (Box and Mumby, 2007; Hughes et al., 2007; Vermeij and Sandin, 2008; Vermeij et al., 2009), sedimentation (Sato, 1985; Babcock and Smith, 2002; Jones et al., 2015; but see Trapon et al., 2013), and direct corallivory (Gallagher and Doropoulos, 2017). Incorporating structural refugia in settlement devices, and controlling the benthic community composition on those surfaces, has the potential to mitigate these stressors and increase the likelihood of survival to size-escape thresholds (Petersen et al., 2005; Nozawa, 2008, 2012; Okamoto et al., 2008; Doropoulos et al., 2012b, 2016; Edmunds et al., 2014; Whalan et al., 2015; Chamberland et al., 2017; Gallagher and Doropoulos, 2017). Optimising the design of microrefugia is challenging, however, due to the complexity of physical (i.e., light availability, sedimentation rates, and flow dynamics) and biological (i.e., benthic competition and herbivory) processes that occur at fine spatial scales around a recruit, and the species-specific responses to those processes. Furthermore, high variability in post-settlement growth rates among species and growth morphologies (Miller, 2014; Suzuki et al., 2018) means that what may work for one species or growth morphology may not work for another.

Larvae of some coral species preferentially settle in crevices and interstitial spaces (Carleton and Sammarco, 1987; Petersen et al., 2005; Whalan et al., 2015), whereas others prefer edges or undersides of substrata (Maida et al., 1994; Babcock and Mundy, 1996; Baird and Hughes, 2000). Yet, strong post-settlement selection in some habitats for corals on upper surfaces (Babcock and Mundy, 1996; Cameron and Harrison, 2020) and those on the undersides closest to the edges (Maida et al., 1994; Cameron and Harrison, 2020) during the first few months suggests that light availability is critically important for driving post-settlement survival in the long-term (Mundy and Babcock, 1998), either directly through the facilitation of growth from photosynthesis, or indirectly through modification of the competitive benthic community on the surfaces around the spat. Consequently, designing artificial seeding devices that provide microrefugia while also maintaining light availability and limiting sedimentation could be advantageous. To that end, we designed and tested the settlement, survival and growth of two deployment device-design types – grooved-tiles and lattice-grids – on aquarium-settled *Acropora tenuis* spat over a ∼1-year deployment on a mid-shelf reef in the central Great Barrier Reef. Our objectives were to describe settlement preferences among the microhabitats within each device design and to compare the survival and growth of spat among device designs and microhabitats to their size-escape threshold in a field deployment.

MATERIALS AND METHODS

Coral Collection, Spawning and Larval Rearing

Gravid *Acropora tenuis* (Dana 1846) colonies were collected from Backnumbers Reef on the central mid-shelf of the Great Barrier Reef (18°30′S, 147° 08′E, GBRMPA Permit G12/35236.1) on the 25th of November 2018, ahead of the predicted coral spawning. *In situ,* colonies were visually inspected for pigmented oocytes in sampled branch tips (Wallace 1985) and then fragments of mature colonies were collected via hammer and chisel and transported to the Australian Institute of Marine Science's (AIMS, Townsville, QLD, Australia) National Sea Simulator (SeaSim[1]). The corals were maintained in temperature controlled outdoor aquaria emulating mid-shelf ambient reef conditions (27.0 ± 0.2°C) and monitored in the evenings for gamete release. On the 28th November, the fifth night after the full moon, the colonies were observed setting gamete bundles in the polyp mouth (Babcock et al., 1986) and were isolated in 60 L tanks. Six colonies synchronously released buoyant egg-sperm bundles at 19:30, approximately 1 h after sunset, and were skimmed from the surface with a clean cup. The bundles were gently agitated and filtered through a 106 μm mesh sieve to separate eggs and sperm. The oocytes were rinsed with 0.4 μm filtered seawater (FSW) and gametes from all six parent colonies were pooled for cross-fertilisation with approximately 10^6 sperm mL^{-1} in a 60 L tank of 4 μm FSW. After 1 h, when embryos were observed cleaving, they were gently rinsed of sperm and transferred into a 500 L flow-through tank for culture. Light aeration was introduced after 24 h (gastrula stage) and was increased after 72 h (swimming planulae) to allow moderate circulation. Larvae were maintained in the culture tank until used in the experiment.

Experimental Device Design

Two types of experimental settlement devices—lattice-grids and grooved-tiles—were designed and manufactured at AIMS to test the effects of various elements of microcrevice design on larval settlement choice and post-settlement survival. The

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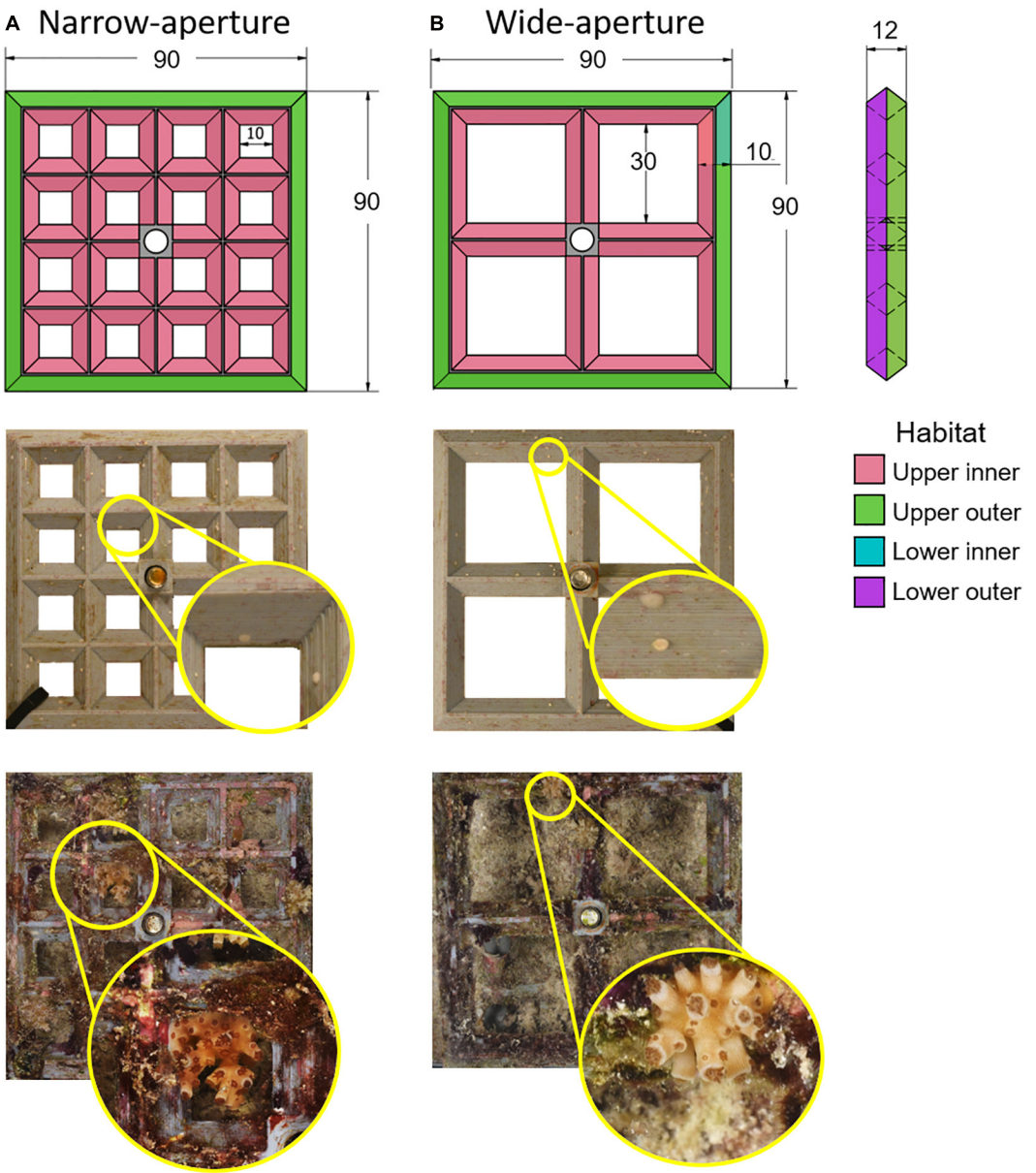

FIGURE 1 | Lattice-grids. Schematic diagrams (top row) with corresponding representative device images immediately after settlement (middle row) and after retrieval from a 1-year field deployment (bottom row) for two lattice-device types: narrow-aperture **(A)** and wide-aperture **(B)**. Colour shading in the first row indicates settlement habitats; note that only upper and outer surfaces are shown in this diagram. Insets for each image in the middle and bottom rows show close-ups of the *Acropora tenuis* spat or juvenile colonies, respectively, on each device type. Each image is of the underside of the device and shows the same corals as spat and juveniles. The brightness and contrast of inset images have been adjusted to improve the discrimination of coral spat and juveniles. In the top row, numbers indicate size in mm.

lattice-grids were 90 mm (L) × 90 mm (W) × 12 mm (H) grey polylactic acid (PLA) plastic 3D-printed grids (**Figure 1**). They had two aperture widths, wide (30 mm) or narrow (10 mm), with 4 × 4 or 2 × 2 grid squares per grid, respectively. On each lattice-grid, four 'habitats' were identified: (i) upper inner, (ii) upper outer, (iii) lower inner, and (iv) lower outer regions (**Figure 1**). We hypothesised that coral recruits on narrow-aperture lattice-grids would be better protected from accidental herbivory damage (hereafter 'grazing') than those on wide-aperture lattice-grids, and also, that 'inner' and 'lower' habitats would be better protected from grazing than 'outer' and 'upper' habitats, due to the protection that microhabitat features can provide from the bites of corallivorous and herbivorous fishes (Gallagher and Doropoulos, 2017). The aperture features

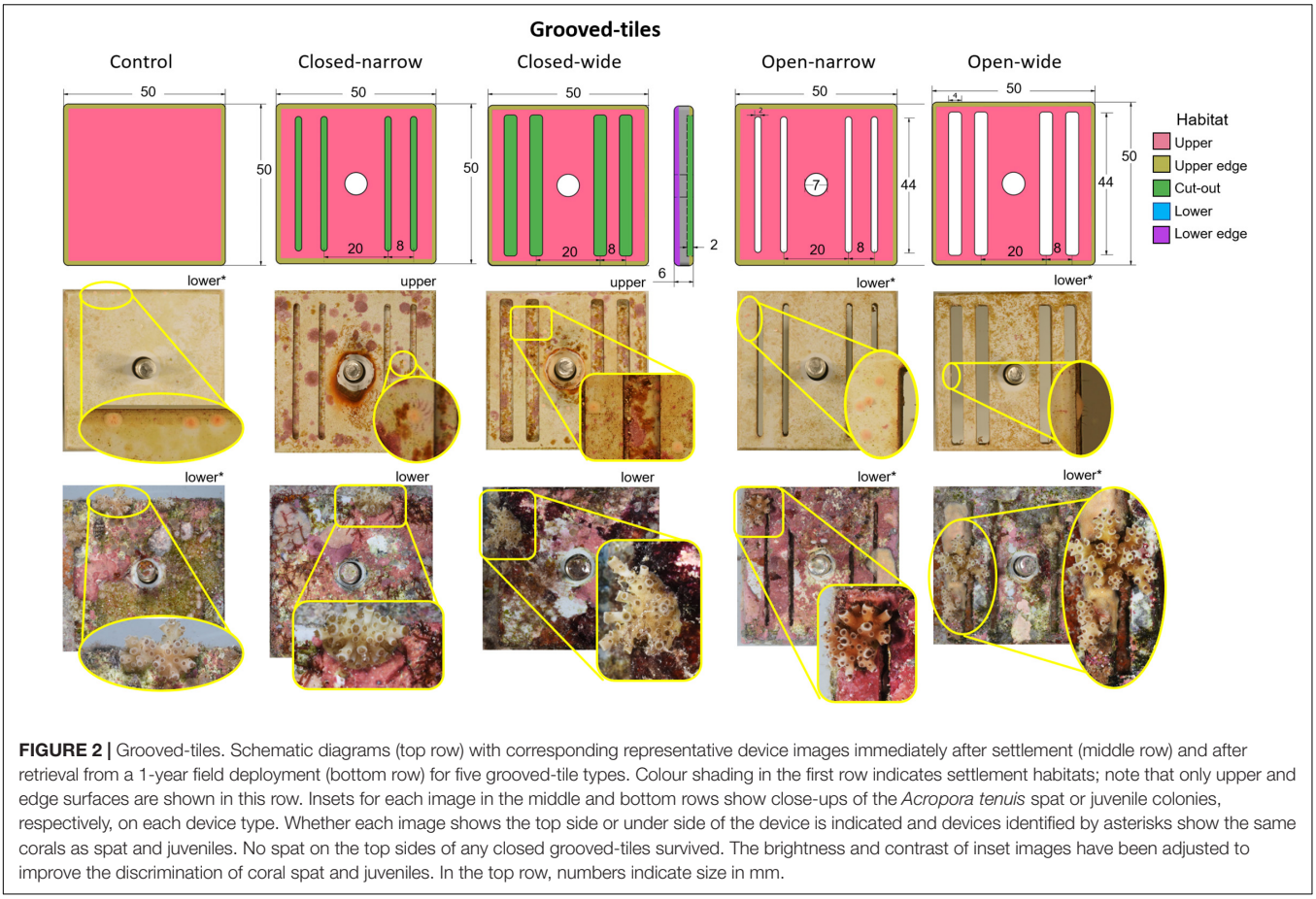

FIGURE 2 | Grooved-tiles. Schematic diagrams (top row) with corresponding representative device images immediately after settlement (middle row) and after retrieval from a 1-year field deployment (bottom row) for five grooved-tile types. Colour shading in the first row indicates settlement habitats; note that only upper and edge surfaces are shown in this row. Insets for each image in the middle and bottom rows show close-ups of the *Acropora tenuis* spat or juvenile colonies, respectively, on each device type. Whether each image shows the top side or under side of the device is indicated and devices identified by asterisks show the same corals as spat and juveniles. No spat on the top sides of any closed grooved-tiles survived. The brightness and contrast of inset images have been adjusted to improve the discrimination of coral spat and juveniles. In the top row, numbers indicate size in mm.

of the lattice-grid devices represent two levels of protection from predation and grazing.

The grooved-tiles were 50 mm (L) × 50 mm (W) mm × 6 mm (H) pieces cut from grey polyvinyl chloride (PVC) sheets (**Figure 2**). Each tile had four grooves and they were either narrow (2 mm) or wide (4 mm) and were either cut all the way through the tile (6 mm deep) to create slits, or were cut 2 mm deep, giving rise to four combinations of grooved-tile types; (i) open-narrow, (ii) closed-narrow, (iii) open-wide and (iv) closed-wide (**Figure 2**). A fifth tile with no grooves served as a control. On each tile, five settlement habitats were classified: (i) upper and (ii) lower faces of the tiles, (iii) within the grooves, and along the (iv) upper edge and (v) lower edge of the bevelled corners (**Figure 2**). We hypothesised that spat settled on the walls of the open grooves would perform better than those settled in closed grooves, due to higher water flow allowing the mass transfer of gases and metabolites (Nakamura, 2010) and a reduced likelihood of smothering from the accumulation of fine sediments (Babcock and Smith, 2002).

Larval Settlement

Prior to settlement, all experimental devices were conditioned in the SeaSim for approximately 4 weeks to develop a biofilm and recruit crustose coralline algae (CCA) for larval settlement induction; all shapes had recruited visible CCA prior to settlement. Acrylic settlement tanks (50 L) with 300 mL min^{-1} flow-through of 4 μm FSW and 112 μm mesh overflow filter, were stocked with either eight replicate lattice-grid devices (four wide and four narrow) or 15 replicate grooved-tile devices (three each of the open-narrow, closed-narrow, open-wide, closed-wide, and control tiles). The lattice-grid devices were each raised slightly (∼1 cm) off the bottom by a central stem and bolt to allow larvae uninhibited access to the device undersides for settlement. Grooved-tile devices were laid directly on the bottom of settlement tanks to promote settlement of larvae in the grooves. There were three replicate settlement tanks for each device experiment, for a total of 24 lattice-grids ($n = 12$ per device type) and 45 grooved-tiles ($n = 9$ per device type).

Eight days after fertilisation, *A. tenuis* larvae were competent to settle, as determined by routine settlement assays in the laboratory (Heyward and Negri, 1999; Nishikawa et al., 2003), and approximately 30 *A. tenuis* larvae per device were added to the tanks for settlement. The larvae were left to settle on the devices for 4 days with 12:12 h light:dark cycle, then relocated to outdoor holding aquaria (2500 L semi-recirculating system, ambient light with 50% shade cloth) on deployment trays. Three replicate deployment trays for each treatment held devices in a raised position approximately 2 cm from the tray bottom, via threaded 316 stainless steel bar through a central hole and secured with a 316 stainless steel wingnut. The distance between adjacent

lattice-grids within a tray was 1–3 cm and the distance between adjacent grooved-tiles within a tray was 2–3 cm.

Upper- and under-side images of the devices were taken 9 and 11 days after larval introduction to the grooved-tiles and lattice-grids, corresponding with approximately 5- and 7-days post settlement, respectively. A cable tie was placed in the corner of each lattice-grid to provide an orientation point for imaging. Pre-deployment images were taken to quantify the number and location of spat settled on each device and habitat; devices were submerged and imaged using a Nikon D810 with a Nikon AF-S 60 mm f/2.8 G Micro ED Lens outfitted with four Ikelite DS160 Strobes mounted on a trolley. For each spat that was mapped, data were recorded on whether that spat resulted from a single larva, or whether an aggregate of larvae had settled together. A spat was classified as an aggregate when there was physical contact between two adjacent larvae at the time of imaging (up to 10 days post settlement). For all subsequent data analyses, aggregated spat were considered as individual recruits. It was not possible to determine whether surviving corals were the result of chimeras or a single, competitively dominant individual, but our focus was on the number of surviving colonies. The trays of devices were maintained in outdoor holding aquaria until deployment.

Deployment and Post-deployment Survival

The lattice-grids and grooved-tiles were transported to Backnumbers Reef (18°29′18.19″S, 147°9′31.31″E) on the 19th December 2018, with spat approximately 1 month old, and deployed onto three replicate fibreglass reinforced plastic (FRP) frames. The deployment site was located on the south-western facing (leeward) side of a northern reef bommie at approximately 6 m depth, in an *Acropora* dominated community (**Supplementary Figure 1**). Each frame [1 m (W) × 1.5 m (L)] was secured with star pickets over rubble substrate immediately adjacent to the bommie and supported the trays of devices approximately 20 cm above the seabed (**Supplementary Figure 1**). Each replicate frame received one tray of lattice-grids and one tray of grooved-tiles.

Survival of *A. tenuis* recruits was tracked by assessment of *in situ* images, taken on SCUBA, of the upper- and under-sides of each device. Images were taken at 41, 95, 185, 246, and 311 days and then imaged again upon retrieval from the field on 16th December 2019 at the final time point of 376 days post settlement. The maximum planar diameter of each live juvenile coral was measured from the final images taken upon retrieval using ImageJ[2]. Detritus and sediments were gently cleared from the devices before imaging. All deployments were undertaken under GBRMPA Permit G18/41046.1.

Statistical Analyses

To compare survival success among device types, within each group of devices (i.e., among grooved-tiles and among lattice-grids), the total numbers of live and dead spat on each device were modelled against device type (fixed effect) using a generalised linear mixed effects model (GLMM), with replicate tray considered as a random effect, using a binomial distribution and a logit-link function. Model assumptions of homogenous variance and normally distributed residuals were verified using the package 'sjPlot' (Ludecke, 2021) and in cases where the overall models were significant, least-squares (marginal) means calculated with a Tukey adjustment were estimated using 'emmeans' to examine pairwise differences in survival response. Because the method of manufacture (3D printing vs. machining slabs), the material (PLA vs. PVC) and the general size and surface texture of the two devices types differed, grooved-tiles and lattice-grids were modelled separately and the two groups of devices were qualitatively compared. All models were run in R (R Core Team, 2020) using the package 'lme4' (Bates et al., 2014) and the data were visualised using the package 'ggplot2' (Wickham, 2016).

To compare survival success among micro-habitats on each device, the total numbers of live and dead spat within each habitat were modelled against habitat type (fixed effect), as described above, with device ID considered as a random effect. We note that the 'upper inner' and 'upper outer' habitats were excluded from the analysis of lattice-grids and that 'upper' and 'upper edge' habitats were excluded from the analysis of grooved-tiles, respectively, because survival success was 0% on these habitat types across all devices.

To evaluate whether there was a relationship between the initial number (and density) of spat settled on each device and the likelihood of having at least one surviving coral on that device after a year, logistic regressions with a binomial distribution and a logit link function were modelled using 'glm' from the 'stats' package in R for each of the device types (R Core Team, 2020). The maximum diameters of the juvenile corals after a year of deployment were also compared among device types using a GLM with a Gaussian distribution, and where necessary, maximum diameter data were log-transformed to meet model assumptions.

To determine whether larvae preferentially settled in certain habitats, Pearson's Chi-squared goodness-of-fit tests were used to compare the observed total counts of spat within each habitat type against the expected numbers of spat in each habitat type, normalised to the surface area of each habitat. Analyses were run separately for each device type. We note that because the grooved-tiles were placed directly on the surface of the tank for settlement, access to the 'lower' habitat may have been partially restricted, despite some settlement on that habitat. We also note that because larvae are known to settle gregariously, it is likely that settlement habitats selected by individual larvae were not strictly independent and this should be considered when interpreting the results of this analysis. The numbers of surviving corals were too low to estimate goodness-of-fit across habitat types after a year of deployment, but the relative proportions of spat that settled within each habitat were qualitatively compared with those that survived through data visualisation.

Finally, to test whether aggregated spat were more likely to survive than single spat, survival data were modelled using a GLMM, as described above, with a binomial distribution and a logit link function. The grouping factor (i.e., single or aggregate)

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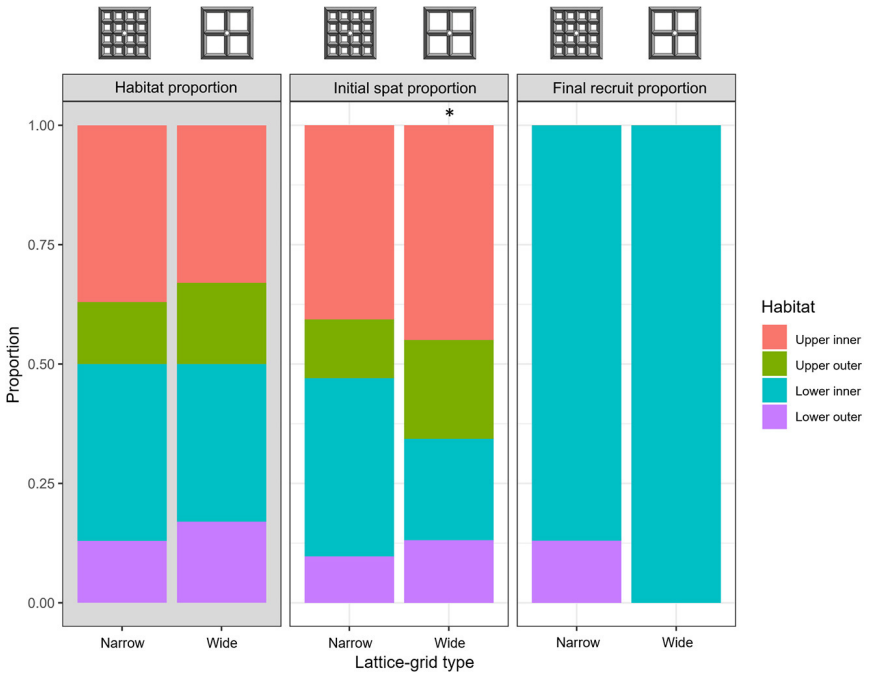

FIGURE 3 | Proportion of space available within each habitat type (grey plot, 'Habitat proportion'), the proportion of live *Acropora tenuis* spat within each habitat type immediately after settlement ('Initial spat proportion') and the proportion of live *A. tenuis* juveniles approximately 1 year post-deployment ('Final recruit proportion') on two types of lattice-grids. Reference diagrams of each device type are included above the plots. Asterisk indicates a statistically significant difference in the observed and expected settlement proportions based on a Chi-square goodness-of-fit test ($p < 0.05$).

was modelled as the fixed effect, and the habitats within each device were treated as random effects.

RESULTS

Lattice-Grids

Larval settlement averaged 38 ± 23 spat (mean ± SD; 0.25 spat cm^{-2}) and 18 ± 20 spat (0.15 spat cm^{-2}) on the narrow and wide lattice-grids, respectively (**Figure 1**). Generally, settlement was highest in the 'upper inner' habitats, followed by 'lower inner,' 'upper outer,' and 'lower outer' habitats, respectively (**Figure 3**). On the narrow aperture lattice-grids, observed settlement was similar to that expected, based on the relative surface area of each habitat ($\chi^2 = 6.2$, df = 3, $p = 0.10$; **Figure 3**). On the wide lattice-grids, however, more larvae settled on the upper surfaces and fewer settled on the lower surfaces than expected, given the available surface area in each habitat ($\chi^2 = 25.1$, df = 3, $p < 0.0001$; **Figure 3**).

Spat survival after 376 days averaged 3 ± 5% (mean ± SD) and 2 ± 3% for the narrow and wide aperture lattice-grids, respectively, with no significant differences in survival detected between narrow and wide types (GLMM: $z = -0.70$, $p = 0.48$) (**Figure 4A**). The spat that survived 376 days were almost entirely located on lower inner habitats (**Figure 3**). No spat survived on any upper surface of either lattice-grid type and were thus excluded from the comparison of survival success among habitat types. Survival was significantly higher for spat on 'lower inner' compared with 'lower outer' habitats across lattice devices (GLMM: $z = -1.97$, $p = 0.049$). A significant rack effect was also observed, such that corals deployed on one rack survived better than those on the other two racks (GLM: $z = 2.826$, $p = 0.005$).

At the device level, 50% of the narrow aperture lattice-grids had at least one surviving recruit, whereas 31% of the wide aperture lattice-grids had at least one survivor after a year (**Figure 4A**). When all lattice-grids were considered together, the likelihood of having at least one survivor on a device significantly increased as a function of the initial number of recruits on that device (GLM: $z = 2.603$, $p = 0.0092$), with the probability of survival switching from favouring 0 (dead) to 1 (alive) at 27 spat (or 0.2 spat cm^{-2}; **Figure 5A**). This result was also significant when the model was run using density data, with survival probability favouring one at 0.2 spat cm^{-2} (GLM: $z = 2.600$, $p = 0.0093$). When each lattice-grid type was considered separately, however, the trends were not statistically significant as the sample size was low.

Approximately 93% of the spat on the lattice-grids were solitary, whereas 7% of the spat resulted from larvae that settled in aggregations of up to 4, with the vast majority of aggregated spat resulting from two larvae settled together (**Figure 6A**). Survival probability on the lattice devices was not dependent on whether larvae settled singly or in aggregates (GMLE: $z = 0.66$, $p = 0.51$), with only one aggregate surviving the full deployment period (**Figure 6C**).

Due to the difficulty of censusing the larger lattice-grids in the field with sufficient resolution to observe recruits, only

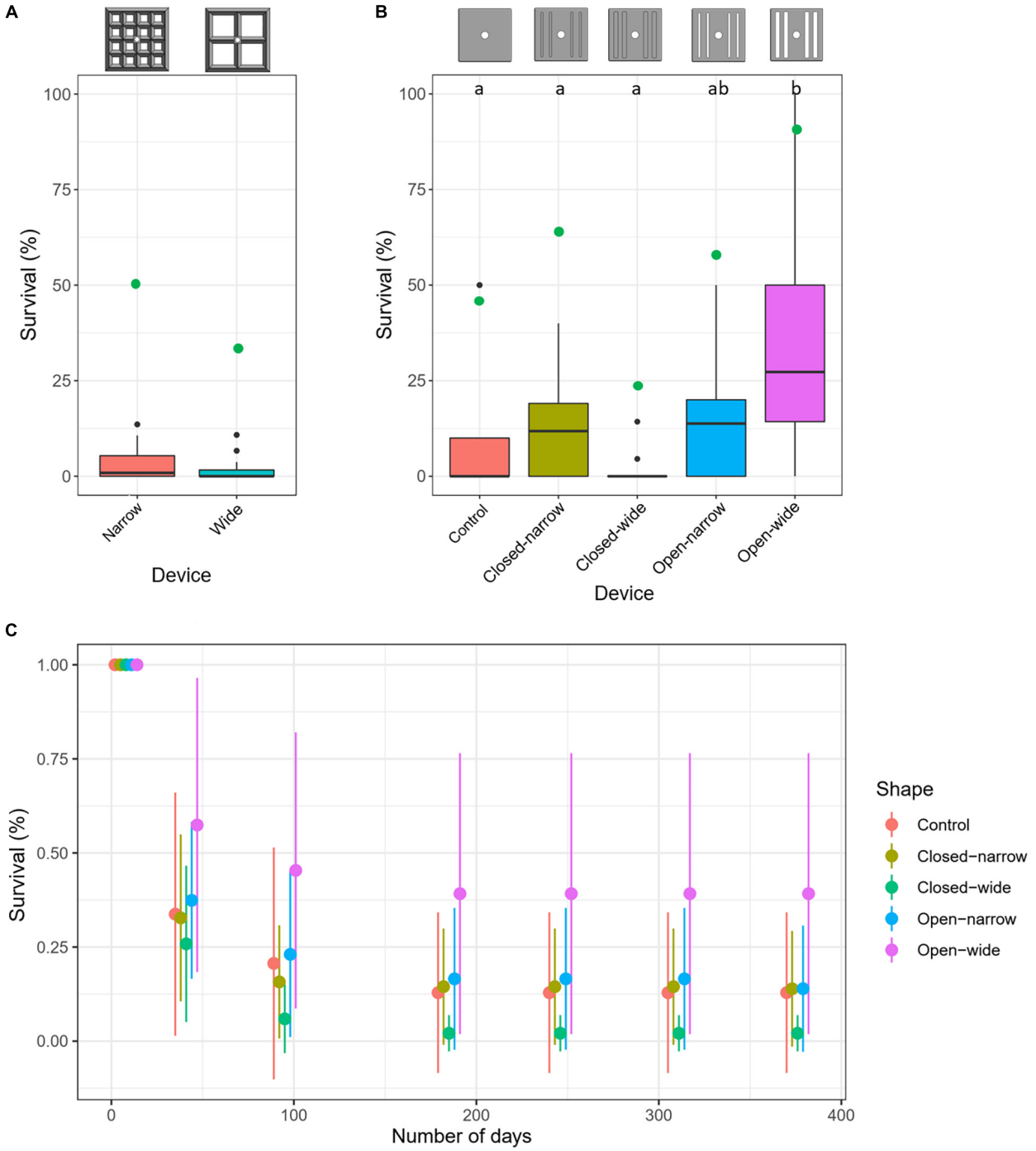

FIGURE 4 | Average survival (%) of *Acropora tenuis* spat after approximately 1 year deployed in the field on two types of lattice-grids **(A)** and on five types of grooved-tiles **(B)**. Letters (a and b) in panel **(B)** indicate statistically significant differences in survival among treatments. **(C)** Average (±SD) spat survival through time on the five types of grooved-tiles. Reference diagrams of each device type are included above the plots in panels **(A)** and **(B)**. Overlaid green points in panels **(A)** and **(B)** indicate survival (%) at the device-level (i.e., having at least one surviving recruit on the device at the end of deployment).

initial and final time points were used in the lattice-device analyses. Qualitatively, however, it appeared that most recruit mortality occurred in the first 3 months post deployment, prior to the first census.

Juvenile corals on the lattice-grids averaged 11.5 ± 4.3 mm (mean ± SD) in maximum diameter after 1 year, ∼20% larger on the narrow aperture lattice-grids (11.9 ± 4.7) than the wide aperture lattice-grids (10.0 ± 2.1), although the

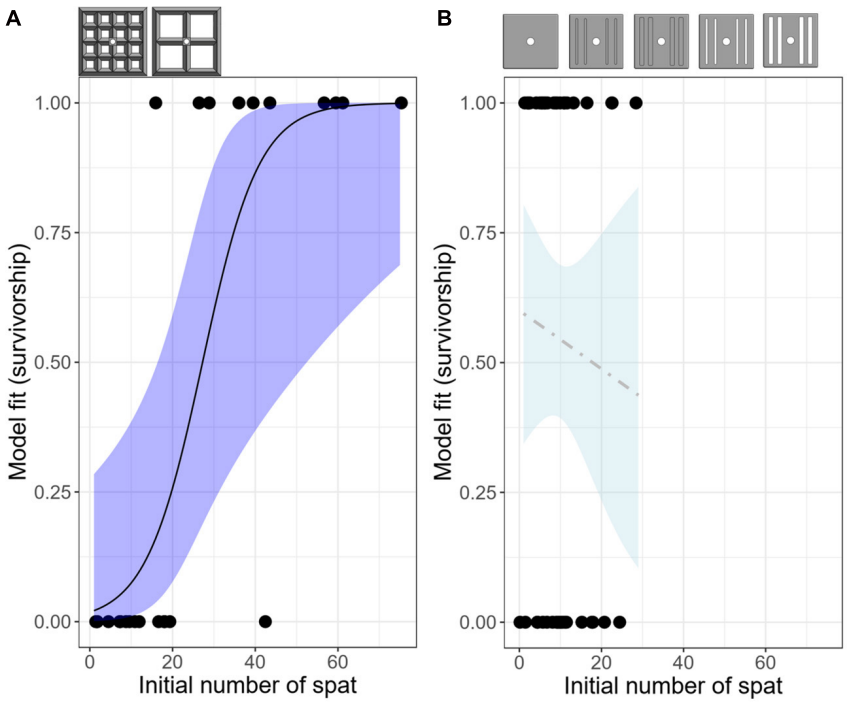

FIGURE 5 | Logistic models predicting the probability of having at least one surviving recruit on each deployed device as a function of the initial number of spat present on that device at the time of deployment, for all lattice-grid **(A)** and grooved-tile **(B)** devices. $n = 24$ for the lattice-grids and $n = 44$ for the grooved-tiles. Shaded areas indicate confidence intervals; dark blue indicates a statistically significant model whereas light blue indicates a non-significant model. Reference diagrams of device types included in each model are above their respective plots.

trends were not statistically significant (GLM: $t = -0.87$, $p = 0.39$).

Grooved-Tiles

Settlement averaged 12 ± 10 spat (mean ± SD; ∼0.25 spat cm^{-2}) per device, and was highest on the closed-narrow grooved-tiles and lowest on the open-wide grooved-tiles, with high variability across device types. In general, settlement was higher in the grooves and on the lower edges than expected, based on available surface area (**Figures 2**, **7**). By contrast, settlement was lower on the upper edges, and lower surfaces than expected, although we note that because the grooved-tiles were placed directly on the surface of the tank for settlement, access to the 'lower' habitat was at least partially restricted, resulting in lower-than-expected proportions of settled spat.

Spat survival averaged 16 ± 24% (mean ± SD) across all devices but was significantly higher on the devices with wide grooves that went all the way through the tile (open-wide, 39 ± 37%) and was lowest on the devices with wide shallow grooves (closed-wide, 2 ± 5%; **Figure 4B**). Surviving spat overwhelmingly were located on lower edge habitats across all device types, except when the grooves went all the way through the tiles; on those devices, spat survived both on the lower-edge habitats and in the grooves (**Figure 7**). No spat survived in any groove that did not go through the tile, and no spat survived on upper or upper-edge habitats on any tile.

The majority of coral mortality occurred within the first 6 months of the deployment (**Figure 4C**). Across all grooved tiles, 55% had at least one surviving recruit after a year, with the highest survival success achieved for the open-wide tile (89%) and the lowest on the closed-wide grooved-tile (22%) (**Figure 4C**). The probability of having at least one surviving spat on a device at the end of the deployment was not significantly predicted by the starting number or density of larvae settled on each device, both when considering all grooved-tiles together and when testing each device individually (**Figure 5B**).

On the grooved-tiles, 90% of settlers were identified as single spat and 10% were classified as aggregates of up to four larvae, with the majority of aggregates composed of two larvae (**Figure 6B**). On the grooved-tiles, aggregations of spat were significantly more likely to survive than single spat (GLMM: $z = -2.3$, $p = 0.02$; **Figure 6D**), although we note that this does not account for the inherent increase in the probability of survival as a function of the number of individuals in an aggregation. There was no clear relationship between the numbers of larvae in an aggregate and the likelihood of that aggregate surviving, with only 10 aggregates surviving after a year deployed.

Juvenile corals that survived a year of deployment averaged 10.4 ± 4.9 mm at the largest, and 8.2 ± 2.5 at the smallest, on the open-wide grooved-tiles and the control tiles, respectively, although the differences in size were not statistically significant.

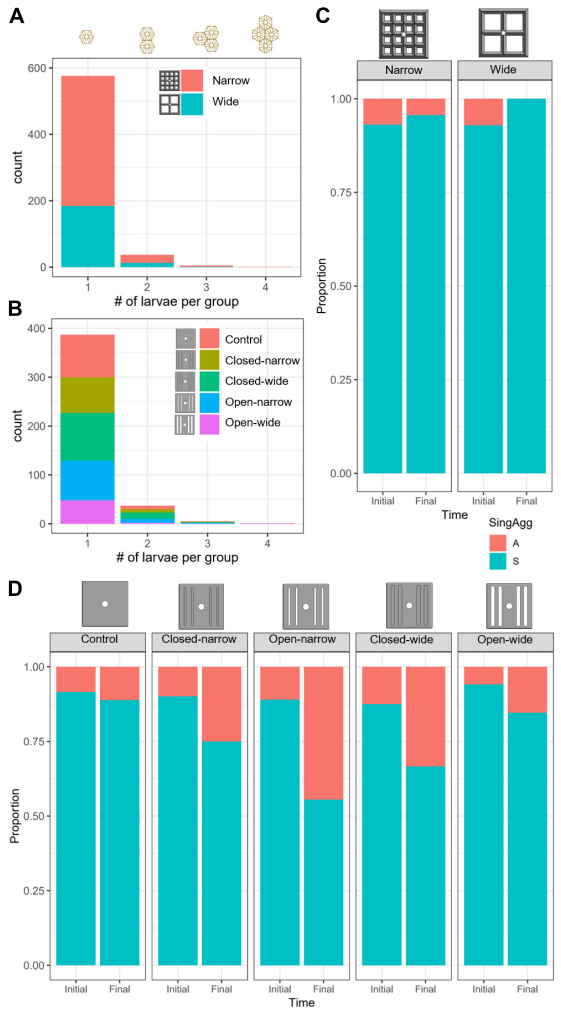

FIGURE 6 | Histograms indicating the distribution of spat that were classified as resulting from 1 (single, "S"), 2, 3, or 4 (aggregated, "A") larvae for the lattice-grid **(A)** and grooved-tile **(B)** devices, by device type (colour). The proportion of spat identified as single spat ("S") or aggregated spat ("A") immediately after settlement (Initial) and after approximately 1 year of deployment (Final) on the lattice-grids **(C)** and the grooved-tiles **(D)**.

DISCUSSION

In this era of coral reef decline (Gardner et al., 2003; De'ath et al., 2012; Hughes et al., 2017), coral populations are fighting an uphill battle toward recovery, contending with reduced cover and a consequent reduction in larval supply exacerbated by Allee effects (Hughes et al., 2019). Of those larvae that do make it to the reef, shifts in the benthic-community composition and the declining condition of the substratum may further impede larval detection of settlement cues, hinder settlement, and reduce post-settlement survival (Albright et al., 2010; Doropoulos et al., 2012a, 2017b; Webster et al., 2013; Fabricius et al., 2015). While difficult to quantify, the mortality of coral settlers can exceed 99% on a healthy reef (Babcock, 1985; Hunt and Scheibling, 1997; Wilson and Harrison, 2005; Vermeij and Sandin, 2008; Penin et al., 2010, 2011; Ritson-Williams et al., 2010; Trapon et al., 2013; Miller, 2014; Suzuki et al., 2018). Seeding reefs with sexually produced coral settlers on deployment devices (Chamberland et al., 2017) is a restoration method with the potential to overcome some of these impediments to recovery, but must achieve unnaturally high levels of post-settlement survival to contend with scalability challenges (Randall et al., 2020). The best performing device tested here, the open-wide grooved-tiles, achieved an average survival of 39% at the level of individual spat—a threefold increase over featureless control tiles—and an even higher 89% at the level of deployment device, suggesting that optimising functional features in deployment devices has the potential to improve spat survival and, consequently, improve the feasibility of larger scale coral seeding.

Patterns of Settlement Within and Among Devices

The settlement patterns observed in this study were complex and somewhat surprising. Coral larvae often preferentially settle in grooves, in microcrevices, and on the undersides of substrates; larvae tend to avoid exposed, upper-facing surfaces (Baird and Hughes, 2000; Petersen et al., 2005; Nozawa, 2008; Vermeij et al., 2009; Whalan et al., 2015; Doropoulos et al., 2016, 2017a; Ricardo et al., 2017; Cameron and Harrison, 2020). While we expected to observe higher settlement on the undersides of the lattice-grids, settlement patterns across those microhabitats roughly reflected the available surface area, suggesting indiscriminate settlement behaviour (**Figure 3**). The reason for this settlement pattern is unknown but several possible explanations warrant consideration. Firstly, there were no horizontal upward-facing surfaces on the devices; instead, the 'upper inner' and 'upper outer' surfaces sloped down at a 51° angle. The lattice devices also did not have dedicated microcrevices to offer alternative settlement locations. Secondly, it could be that, because settlement took place in experimental aquaria under artificial lights, the environmental drivers of preferential settlement on downward-facing surfaces, such as natural light gradients (Maida et al., 1994; Mundy and Babcock, 1998; Baird and Hughes, 2000) and sedimentation gradients (Jones et al., 2015; Ricardo et al., 2017) were lacking. Thirdly, because the devices were conditioned in the laboratory and for only 4 weeks, the differential biological communities typical of those microhabitats may have been less pronounced at the time of settlement and different from field-conditioned communities (Doropoulos et al., 2017b) although they were markedly different among microhabitats by the end of the deployment period (**Supplementary Figure 1**).

Settlement behaviour on the grooved-tiles was difficult to assess due to the positioning of tiles directly on the base of the tank to promote settlement in the grooves, which at least somewhat restricted access to the lower surface. However, there was some selective settlement behaviour—larvae avoided settling on the upper edges of the tiles but settled in all other available microhabitats including upward-facing horizontal surfaces, within the grooves, and on lower edges (**Figure 7**).

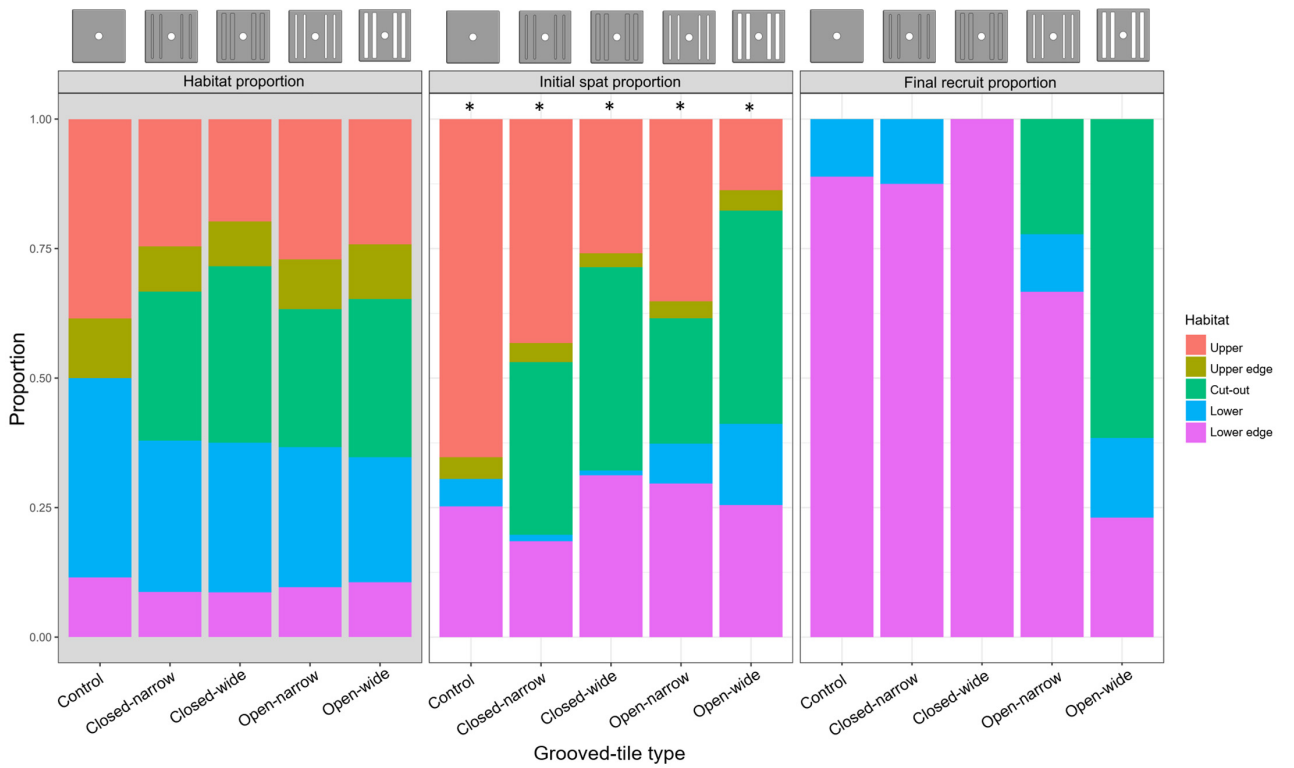

FIGURE 7 | Proportion of space available within each habitat type ('Habitat proportion'), the proportion of live *Acropora tenuis* spat within each habitat type immediately after settlement ('Initial spat proportion'), and the proportion of live *A. tenuis* juveniles approximately 1 year post-deployment ('Final recruit proportion') on five types of grooved-tiles. Note that the control devices did not have any grooves. Reference diagrams of each device type are included above the plots. Asterisks in the 'Initial spat proportion' data indicate statistically significant differences in the observed and expected settlement proportions based on a Chi-square goodness-of-fit test ($p < 0.05$), but we stress that access to the 'lower' habitat by the larvae was at least partially restricted.

Again, the relatively high proportion of spat settling on upward-facing surfaces was surprising but could be due to the immature biological communities on the tiles or the environmental conditions present at the time of settlement.

Given the strong patterns observed in recruit survival, detailed in the next section, investigating ways to direct settlement to the microhabitats that confer the highest survival may further improve the performance of the devices. This could be achieved by modifying the environmental conditions present at the time of settlement (i.e., light or flow), restricting access to upward-facing surfaces during settlement (i.e., using physical barriers or antifoulants), modifying the duration of, or conditions for, benthic-community development prior to settlement, or promoting particular benthic species to induce settlement (i.e., promoting particular crustose coralline algae species).

Patterns of Survival Within and Among Devices

Spat survival averaged between 2 and 39% over 376 days, depending on the device design, and declined most rapidly within the first 6 months, typical of survival on artificial deployment devices (reviewed in Randall et al., 2020). Spat survival is affected by a myriad of factors, which make comparisons of spat survival among studies difficult to interpret. Nevertheless, average spat survival on the open-wide grooved-tiles (39%) was high compared with what has been achieved in similar deployment studies over an equivalent time frame (12 months), which tend to average <10–20% (Chamberland et al., 2015, 2017; dela Cruz and Harrison, 2017, 2020; Baria-Rodriguez et al., 2019). Small variations in the holding period prior to deployment can have significant downstream consequences for survival estimates, however, and thus we avoid making direct comparisons with other studies. Comparing spat survival on artificial surfaces against natural substrates is also difficult, since the larvae often settle in cryptic locations and are extremely difficult to locate *in situ* during the first 6 months (dela Cruz and Harrison, 2017, 2020). Therefore, our comparisons of spat survival below focus on the factors (i.e., microhabitat features and device designs) that could be quantitatively compared under the deployment conditions.

In this study, spat mortality on the upper surfaces and upper edges was 100% across all device types; the vast majority of surviving recruits were located on lower-edge microhabitats (**Figures 3**, **7**) although many of the survivors had begun to grow up around the sides of the devices by the end of the deployment (**Figures 1**, **2**). We hypothesize that these results

were primarily driven by processes related to sedimentation, light attenuation, and grazing.

Firstly, no spat survived in groove microhabitats when they were closed, but the open-wide grooves were the best performing microhabitats (**Figure 7**). Observations during field deployments indicated that sediment accumulated in the turfs within the grooves when they were not cut all the way through the tiles whereas the open grooves appeared to reduce sediment load (Jones et al., 2015; Ricardo et al., 2017). Interestingly, the grooves seemed to harbour a turfing community (**Supplementary Figure 2**), perhaps due to restricted herbivory, but the spat were better able to contend with the turf when sediment did not accumulate.

Secondly, survival was higher on the lower inner microhabitats compared with the lower outer microhabitats on the lattice-grids, a result similar to that of Gallagher and Doropoulos (2017) who found that inner crevices protected coral juveniles better than outer crevices. This may be because the inner areas of the lattice-grids had reduced access to herbivores but still allowed the interior underside habitats to function more like 'edge' habitats, by allowing light to pass through the lattice-grid and reach the recruits. Maida et al. (1994) documented that mortality of coral spat on the undersides of settlement plates increased with distance from the edge of the plate and attributed this to the decay of light intensity from the outer edge to the centre of the tiles. Indeed, older coral juveniles are often found on exposed edges and upper surfaces reflecting the strong role of light in driving juvenile and adult coral distributions (Maida et al., 1994; Babcock and Mundy, 1996; Baird and Hughes, 2000; Cameron and Harrison, 2020). For example, Cameron and Harrison (2020) documented the proportional change in *A. tenuis* distribution on settlement tiles over 12 months during a field deployment. Spat were overwhelmingly dominant on underhangs 5 days after settlement but were located almost entirely on the edges of the tiles after 12 months, with more than half of the surviving corals having originally settled on underhangs and subsequently grown onto the edges. We suggest that the wide grooved-tiles and wide lattice-grids promote edge-like conditions throughout the undersides of the shapes. Indeed, there were no differences in the maximum diameter of recruits among microhabitats or device-types in this study, suggesting no obvious limitations to growth (**Supplementary Figure 3**).

Lastly, it was clear that upper surfaces of the grooved-tiles were heavily grazed by fish, based on field observations of roving acanthurid and scarid schools, the presence of resident pomacentrids, and *in situ* images that show an abundance of bite marks (**Supplementary Figure 2**). We hypothesize that this herbivory pressure removed the spat on upper surfaces where fish had access (Penin et al., 2010; Trapon et al., 2013). Indeed, Trapon et al. (2013) undertook an herbivore exclusion experiment of *Acropora cytherea* recruits on the Great Barrier Reef and found that the exclusion of herbivores significantly increased survival of recruits on the reef crest.

While we documented clear patterns in post-deployment survival of *Acropora tenuis* among microhabitats on the devices, these patterns may vary among environments with differing light and flow regimes, across depth gradients and among species. For example, Baird and Hughes (2000) documented a significant difference in recruitment of *A. hyacinthus* in shaded and unshaded environments owing to differential light levels and Miller (2014) identified species-specific responses to deployment orientation in *A. palmata* and *Orbicella faveolata* in the Caribbean. Patterns of recruit survival are also likely to differ across ontogeny as the relative influence of various intrinsic and environmental pressures shift (Babcock and Mundy, 1996; Doropoulos et al., 2017a). For example, Babcock and Mundy (1996) identified divergent drivers of mortality during the first 4 months after settlement compared with the subsequent 5 months, suggesting that there are competing pressures that vary in their relative influence through time, and indicating that the method of deployment should consider all the drivers of mortality throughout early development to optimise survival success.

Density Dependence in Spat Survival

Relationships between spat density and recruit survival have been described for several *Acropora* species and suggest that intermediate densities often confer the best outcome (Suzuki et al., 2012; Edwards et al., 2015; Doropoulos et al., 2017a, 2018; Cameron and Harrison, 2020). Suzuki et al. (2012) examined the impact of larval density in a field seeding trial of *A. tenuis* and *A. muricata* in Japan and found that moderate spat densities ($\sim$0.1 cm^{-2}) resulted in a better overall outcome than low ($\sim$0.07 spat cm^{-2}) and high densities ($\sim$0.6 cm^{-2}), as determined by survival at 6 months and genetic diversity endpoints. Similarly, in a 2-year field study of *Acropora tenuis*, Cameron and Harrison (2020) found that the highest colony abundance and coral cover was achieved on tiles with an intermediate spat density ($\sim$1 cm^{-2}), although the intermediate density was higher than that described by Suzuki et al. (2012), perhaps owing to the differences in the richness of species deployed and the environmental conditions at the deployment sites. Similarly, here we identified a significant increase in the likelihood of having at least one surviving coral on a lattice device when there were at least 0.2 spat cm^{-2}. The highest settlement density achieved in the study was 0.5 spat cm^{-2} and no negative density-dependent effects were observed. The mechanisms that drive these density-dependent effects, and the variations in those effects observed within and among studies (i.e., Doropoulos et al., 2017a) are complex but likely include space limitation leading to inter- and intra-specific competition and more settlement in sub-optimal microhabitats as density increase (Roughgarden et al., 1985; Cameron and Harrison, 2020), mediated by genetic relatedness of the spat (see next section). Regardless of the mechanism, our results support these previous findings and suggest a minimum density of at least 0.2 spat cm^{-2} for *A. tenuis* seeding devices.

Restoration Outcomes With Chimeras

Coral larvae tend to settle gregariously and may occasionally form chimeras, which can increase juvenile survival, size and growth (Raymundo and Maypa, 2004; Amar et al., 2008; Suzuki et al., 2012; dela Cruz and Harrison, 2017; Doropoulos et al., 2017a). Promoting gregarious settlement on seeding devices has been proposed as a method for improving restoration outcomes

(Raymundo and Maypa, 2004; Puill-Stephan et al., 2012b) but its usefulness will depend on the level of allorecognition and genetic histocompatibility in the aggregation (Puill-Stephan et al., 2012a,b; reviewed in Randall et al., 2020). Furthermore, the benefit of aggregated settlement is context dependent, and can vary in response to environmental factors and spat settlement location (Doropoulos et al., 2017a). For example, Doropoulos et al. (2017a) identified a positive effect of gregarious settlement on survival of spat on exposed surfaces but not of those that settled in crevices. In this study, the impact of aggregated settlement on the likelihood of survival was also mixed. On the one hand, there were no differences in survival probability between single and aggregated spat on the lattice devices, while on the other hand, aggregations of spat were more likely to survive on the grooved-tiles. However, these analyses compare single and aggregated spat survival directly, and do not account for the inherent increase in the probability of survival as a function of the number of individuals in an aggregation. In other words, there are more 'chances' for the coral to survive as the number of spat in an aggregation increases. Therefore, these results likely overestimate the benefit of aggregation in spat survival.

The present study evaluated spat from six contributing parents of one species, which likely led to a large proportion of closely related (half and full sibling) spat. While histocompatibility mechanisms have not been investigated in *Acropora tenuis*, Puill-Stephan et al. (2012b) found that the expression of allorecognition in the similar species *A. millepora* took at least 5 and 13 months for half- and full-siblings, respectively, suggesting that our deployment of 376 days may not have been long enough to capture the full allorecognition response in the spat. Thus, it is possible that rejection within some chimeras was yet to take place. Furthermore, chimeric adult *A. millepora* genotypes show high levels of relatedness suggesting that genetic similarity is required for long-term persistence of the chimera (Puill-Stephan et al., 2009). Thus, while we documented an increase in survival probability of aggregated spat on the grooved-tiles, it may not be advantageous to promote gregarious settlement of spat when a more diverse broodstock contributes to a mass culture used in restoration. We suggest that longer-term research on more genetically diverse spat and on more species is needed to determine the value of promoting aggregated settlement for restoration.

Limitations

While we documented a clear improvement in spat survival associated with specific device-design features, the mechanisms driving this response remain unknown. We hypothesise that protection from fish-feeding behaviours (Trapon et al., 2013), prevention of sediment accumulation (Sato, 1985), and improved light attenuation all may have contributed to improved device function. Yet ascertaining the likely non-linear direct and indirect effects of these features on the mechanisms driving survival requires additional experiments, particularly with devices directly deployed onto the reef framework. Due to permitting limitations, the devices were deployed on fibreglass frames immediately adjacent to (within 1 m of) the bommies over a rubble and sand bottom. Anecdotal observations made during the deployment and census trips suggest that the frames attracted resident and transient grazing fishes, potentially subjecting the shapes to disproportionally high grazing pressure. Yet other strongly site-associated organisms may not have ventured out to the racks, also potentially altering the natural grazing community. Seeding devices directly onto the reef would remove this potential bias and improve our understanding of the drivers of post-deployment survival.

Three-dimensional (3D) printed plastic shapes are cheap and easy to produce to test various design features and to use as proxies for future deployment devices. However, the biofilms and biological communities that develop on plastics during conditioning are different from those on terracotta, ceramic and other materials and can have downstream consequences on benthic community composition, grazing, and microenvironments (Kennedy et al., 2017). The design features tested with 3D printed plastic require validation with acceptable materials for use in reef restoration (Spieler et al., 2001; Randall et al., 2020).

CONCLUSION

Optimising functional features in deployment devices can improve spat survival and may consequently support the up-scaling of coral seeding. Doubling the survival rate allows for halving the scale of deployment to achieve the same restoration goal, and could potentially lead to substantial reductions in cost per unit area, acknowledging that restoration costs do not scale linearly with restored area (Anthony et al., 2019; Gibbs et al., 2019). In this study, the wide-grooved-tiles achieved three times higher average survival than the comparatively featureless tiles of the same material, showing promise for overcoming bottlenecks of survival to size-escape thresholds and improving scalability. Incorporating grooves in other recently designed deployment substrates, such as 'coral plug-ins' (Guest et al., 2014; Tabalanza et al., 2020) and tetrapods (Chamberland et al., 2017) may improve survival and warrant further testing. While the wide grooved-tiles worked best for the *Acropora tenuis* spat tested here, it is likely that the drivers of mortality differ among species, among coral growth morphologies, and throughout ontogeny; identifying what design features work best in these various scenarios and across environmental gradients requires further investigation. One approach to device design could be to integrate multiple design features into a single device to maximise its effectiveness across a broad cross-section of hermatypic corals. Another approach could be to develop a suite of designs fit for particular growth morphologies, taxa, and receiving environments. A third approach could be to uncouple the settlement device from the deployment device. In this scenario, a small device could be designed with microrefugia, such as wide grooves, to direct settlement and enhance post-settlement survival. The settlement device would then be attached in modular fashion to a larger deployment device designed to optimise deployment logistics while maximising survival and retention on the reef. Regardless of the approach, innovative

and fit-for-design devices (Petersen et al., 2005; Nozawa, 2008; Okamoto et al., 2008; Chamberland et al., 2017) may improve restoration outcomes in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CR, AN, and AH conceived of and designed the experiments. CR and CG conducted the experiments. CR analysed the data. CR and CG wrote the manuscript. All authors edited and approved the final draft.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.662263/full#supplementary-material

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Selecting Heat-Tolerant Corals for Proactive Reef Restoration

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Coral reef restoration is an attractive tool for the management of degraded reefs; however, conventional restoration approaches will not be effective under climate change. More proactive restoration approaches must integrate future environmental conditions into project design to ensure long-term viability of restored corals during worsening bleaching events. Corals exist along a continuum of stress-tolerant phenotypes that can be leveraged to enhance the thermal resilience of reefs through selective propagation of heat-tolerant colonies. Several strategies for selecting thermally tolerant stock are currently available and range broadly in scalability, cost, reproducibility, and specificity. Different components of the coral holobiont have different utility to practitioners as diagnostics and drivers of long-term phenotypes, so selection strategies can be tailored to the resources and goals of individual projects. There are numerous unknowns and potential trade-offs to consider, but we argue that a focus on thermal tolerance is critical because corals that do not survive bleaching cannot contribute to future reef communities at all. Selective propagation uses extant corals and can be practically incorporated into existing restoration frameworks, putting researchers in a position to perform empirical tests and field trials now while there is still a window to act.

Keywords: coral bleaching, thermal tolerance, selective propagation, climate change, restoration

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INTRODUCTION

Like many natural resources, coral reefs are facing the consequences of climate change. Regardless of contemporary reductions in emissions, Earth is committed to several degrees of warming (Sherwood et al., 2020) that will impact a wide range of ecosystems. Ocean warming is causing increasingly frequent and severe thermal stress events on coral reefs, triggering bleaching that results in physiologically and metabolically impaired corals. When corals become so severely compromised that they are unable to recover from temperature stress, reef ecosystems degrade with immediate and latent consequences (Hughes et al., 2018). Climate change has already caused dramatic losses in coral cover worldwide (Wilkinson, 2004). Without intervention, the rate of change in environmental conditions will likely soon outpace natural flexibility and adaptive capacity (Bay et al., 2017; Matz et al., 2018) to maintain functional coral reefs and the ecosystem services they provide. The serious implications for food security, coastal protection, and biodiversity have compelled the search for active management solutions and expanded interest in reef restoration projects (van Oppen et al., 2017).

Resource managers are increasingly aware that win-win outcomes that conserve biodiversity and maintain human interests may be impossible (McShane et al., 2011). Many research groups and management agencies are calling for immediate exploration of non-conventional management

strategies and dramatic human interventions for coral reefs while they can still be effective (Hardisty et al., 2019; National Academies of Sciences Engineering and Medicine, 2019; Anthony et al., 2020). Strategies involving reef restoration are attractive because they offer a direct intervention using physical and logistical techniques that are already established. However, under climate change, conventional restoration approaches must be modified to be more proactive, accounting for future conditions, because restoration using coral stock that is intolerant of climate change will likely be inefficient and ineffective.

These proactive restoration efforts must be conceptually reasonable, have manageable or scalable risk, and be logistically feasible. Here we argue that coupling coral propagation and reef restoration practices with methods for identifying heat-tolerant corals meets these criteria and should be assiduously explored. Selective propagation of local thermally tolerant coral stocks uses existing corals and techniques, can be readily integrated with ongoing restoration programs, and theoretically enhances the temperature resilience of the outplant site. To establish the efficacy of the approach while it still has relevance, field-testing should be performed now.

AIMS AND PRACTICES OF CORAL REEF RESTORATION

Ecological restoration is defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" and a successfully restored ecosystem "contains sufficient biotic and abiotic resources to continue its development without further assistance or subsidy" (Society for Ecological Restoration International Science and Policy Working Group, 2004). Since scleractinian corals are the foundation of the coral reef ecosystem, interventions to increase the amount of living hard coral cover are a primary focus in reef restoration projects (Precht and Robbart, 2009), which typically target degradation traceable to anthropogenic activities for which mitigation measures exist (e.g., ship grounding, dredging, localized runoff). Coral reef restoration is still an emerging field undergoing technological and conceptual innovation (Omori, 2019). Improving restoration techniques and furthering ecological knowledge have been the main motivations for reef restoration projects over the past several decades (Bayraktarov et al., 2019) and best practices are emerging with the lessons learned.

The majority of coral reef restoration projects currently involve direct outplanting of whole or fragmented corals chosen opportunistically and transplanted. Fragmentation of hard corals was pioneered and developed by the commercial aquarium trade (Delbeek, 2001) and is utilized extensively by reef restoration practitioners to asexually propagate coral stock for transplantation (Rinkevich, 2014; Boström-Einarsson et al., 2020). The "coral gardening" approach has adapted this technique to include an intermediate nursery phase (either *in situ* or *ex situ*). Coral gardening allows time for fragments to recover and grow to an adequate size before outplanting (Rinkevich, 1995, 2005) and represents a "sustainable" source of material for restoration that minimizes continuous harvest from the broader population.

Sexual propagation of corals is also being explored as a potential restoration tool either through directly seeding reef areas with larvae (Doropoulos et al., 2019; Cameron and Harrison, 2020) or using settled spat (from controlled crosses or large-scale wild spawning events) to obtain stock for outplanting (Nakamura et al., 2011; Villanueva et al., 2012). Sexual reproduction allows selective breeding of particular traits and increased genotypic diversity in wildtype crosses, which may improve adaptive and evolutionary potential (Bay et al., 2017; Quigley et al., 2020a). The vast numbers of coral larvae resulting from spawning events and their small individual size means sexual reproductive approaches have the potential to be scaled up in ways that fragmentation cannot. However, sexual reproduction is less tractable than asexual propagation because approaches require more extensive effort and expertise, can vary widely in methodology by species, and are often dependent on seasonal events.

Sexual or asexual approaches to obtaining source material for outplanting could be combined with other proposed restoration strategies (e.g., artificial or augmented substrates meant to convey a settlement or growth advantage to desirable species, thermal preconditioning, heterotrophic feeding, probiotics) to gain synergistic benefits. Regardless of the particular strategy, theoretical consideration of the techniques, consequences, and limitations is crucial to the effectiveness of any restoration project. Practical concerns of resource managers and stakeholders, such as preserving ecosystem services, maintaining biodiversity, retaining or increasing coral cover, and preventing phase shifts, necessitate the ongoing development of restoration techniques.

CORAL REEF RESTORATION UNDER CLIMATE CHANGE

Climate change presents a major challenge to traditional resource management because increasing atmospheric CO_2 is a pan-global driver whose mitigation is outside the purview of any single resource management agency. The inevitability and enormity of the problem has led resource managers and stakeholders to reconsider traditional conservation goals and to start planning for climate change adaptation—managing change rather than maintaining conditions (Palmer et al., 2004; Stein et al., 2013). Climate change is increasingly considered in forestry management planning and the control of terrestrial invasive species (Nagel et al., 2017; Beaury et al., 2020) and interest in resilience-based management of coral reefs is growing (Mcleod et al., 2019).

While it has been assumed that local management actions help mitigate coral bleaching effects by reducing additive and synergistic stressors (Anthony, 2016), recent evidence suggests that recurring incidences of more extreme heat stress may limit the benefits (Hughes et al., 2017). In the past 50 years, approximately 50% of the Great Barrier Reef (Dietzel et al., 2020) and >80% of the Caribbean (Gardner et al., 2003) has been

degraded. It is estimated that only 10% of the world's reefs will persist past the year 2050 (Burke, 2011), as bleaching becomes a nearly annual occurrence (van Hooidonk et al., 2013). Even under best-case emissions trajectories, coral reefs will continue to be negatively transformed by climate change (Hughes et al., 2018; Anthony et al., 2020).

Conventional coral reef restoration is unsuitable under climate change because increasing temperature stress must now be accepted as an established parameter of the environment which will continue to impact newly outplanted corals during restoration (Drury et al., 2017a; Drury and Lirman, 2021). Without the introduction of meaningful adaptive variation there is a mismatch in the speed of adaptation relative to climate change (Matz et al., 2020), leading to local extirpation and limiting the long term persistence of reefs (Bay et al., 2017). Returning a degraded coral reef to its pristine state was once a realistic goal, but in many locations it now appears that priorities must shift to supporting ecosystems that are more resilient to climate change even if they represent modified versions of the ideal state. Fortunately, the existing coral restoration toolbox is diverse and potentially adaptable to proactive restoration objectives (Rinkevich, 2019).

PROACTIVE CORAL REEF RESTORATION

The terms "proactive restoration" and "preemptive restoration" appear occasionally in terrestrial resource management literature (Schweitzer et al., 2014; Muzika, 2017; Schoukens, 2017; Schweiger et al., 2018) especially in fields where there are strong anthropocentric concerns, such as endangered species compliance or timber management. In *Foundations of Restoration Ecology* (Falk et al., 2006), the authors state, "By proactive, we mean restoration projects that are designed to accomplish more than returning a system to some prior state." Perhaps the term is not used more frequently, despite the concept being evident in many studies, because there is already a foregone conclusion in terrestrial systems that we will be factoring climate change into the design of management plans for the foreseeable future. We suggest the term "proactive restoration" is applicable to coral reef restoration undertaken in anticipation of environmental change and accounting for expected future conditions.

We advocate combining methods for identifying heat-tolerant coral stock with existing best practices in propagation and outplanting as a viable proactive reef restoration strategy that should be explored in earnest. Using coral stock selected to persist under anticipated future climate conditions should enhance the long-term survivorship of the individual outplanted colonies and consequently reduces wasted effort by practitioners. Transplantation of thermally tolerant individuals can also support adaptation (Bay et al., 2017), with models that include migration and selection for optimal genotypes predicting coral reefs in specific geographic ranges could persist for 100–200 more years (Matz et al., 2020).

Our focus is on the propagation of heat-tolerant colonies selected from a local population for use in restoration projects in the same locale, but less conservative formulations are also possible. Practitioners faced with insufficient thermal-tolerance in a local coral population could consider applying a "climate adjusted provenancing" approach (Prober et al., 2015; Baums et al., 2019) that includes selecting heat-tolerant corals from more distant reefs, representing a hybrid strategy of assisted gene flow (Aitken and Whitlock, 2013) and selective propagation. In contrast to the conventional objective of repairing damage, selective propagation and outplanting could hypothetically be implemented prior to any evidence of degradation in order to preemptively support resilience of coral populations in anticipation of an imminent decline. While integrating thermal resilience in coral populations using this proactive restoration approach is largely untested and subject to issues of scale, reef restoration without climate change planning is already untenable.

PERSISTENCE OF HEAT-TOLERANCE

Many strategies proposed for human intervention in coral conservation involve moving individual corals along with their algal, bacterial, and viral symbionts, taking advantage of intrinsic adaptive variation within and among populations (**Figure 1**). Proactive restoration using selected heat-tolerant coral stock requires that the extant thermal tolerance available in local coral populations is sufficient to persist under more stressful future conditions and that propagated individuals retain a significant portion of the heat-tolerance identified in source colonies. Multiple components of the coral holobiont drive phenotypes of interest, but their utility for practitioners may vary.

Genetic drivers of thermal tolerance in the coral host are well-supported by experimental evidence on heritability (Dixon et al., 2015; Kirk et al., 2018), the long-term persistence of thermal tolerance after acclimatization (Schoepf et al., 2019), transplantation (Palumbi et al., 2014; Kenkel and Matz, 2016), environmental correlates (Jin et al., 2016), and reproducible bleaching effects (Ritson-Williams and Gates, 2020; Voolstra et al., 2020). This evidence suggests that host genetic effects have the highest translatability of any component of the holobiont and are most useful for practitioners, despite our limited understanding of genotype by environment interactions (Howells et al., 2013; Drury and Lirman, 2021). The utility of host genetic effects does not require any actual data on genomic variants, but can be established through broad-sense (clonal) heritability by measuring phenotype(s) of known individuals.

Corals can also harbor multiple genera of *Symbiodiniaceae* simultaneously (Silverstein et al., 2012), which can shift in response to natural and experimental heat stress (Baker et al., 2004; Berkelmans and van Oppen, 2006; Cunning et al., 2015b). However, different coral genera have varying levels of tolerance for diverse symbiont assemblages and flexibility in symbiont associations may be genera specific or temporally unstable (Goulet, 2006; Thornhill et al., 2006). Conversely, some corals bleach and recover without shuffling symbionts (Cunning et al., 2016) and recapitulate stress tolerance phenotypes through multiple bleaching events (Fisch et al., 2019; Ritson-Williams and Gates, 2020). There are also fine-scale differences within

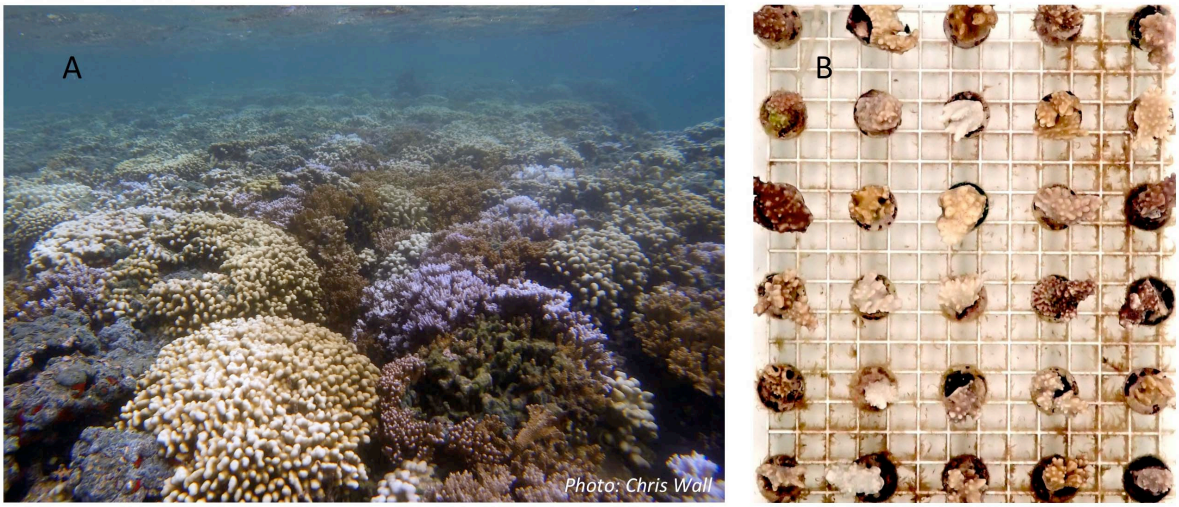

FIGURE 1 | Variation in the response of corals to heat stress. **(A)** Colonies of several species on a reef during a bleaching event. **(B)** Coral fragments of a single species undergoing an artificial aquarium-based heat stress test.

symbiont genera (Sampayo et al., 2008) and potential genotype level physiological implications (Baums et al., 2014). We suggest that in certain instances symbiont community dynamics may be a translatable factor that influences phenotype in a useful manner for practitioners, but additional data on historical, environmental, and species-specific factors is needed.

The evidence for bacterial translatability is equivocal. Temperature stress is associated with shifts in the microbiome (Bourne et al., 2008; Littman et al., 2011) and corals with more stable microbiomes tend to be more thermally tolerant (Hadaidi et al., 2017; Grottoli et al., 2018). Specific bacteria (Ben-Haim et al., 2003; Thurber et al., 2009; Mouchka et al., 2010) are correlated to bleaching response and specific bacterial functions (Santos et al., 2014) have also been linked to thermal tolerance. Bacterial probiotics used to supplement microbial communities of corals can improve thermal tolerance in laboratory experiments (Rosado et al., 2019). Conversely, corals from different thermal environments have unique microbes that may shift when moved to more stressful environments (Ziegler et al., 2017) and bacterial communities are flexible during development, aging, and bleaching (Littman et al., 2011; Williams et al., 2015; van Oppen and Blackall, 2019). Although the microbiome does play a role in thermal tolerance, the complexity of this component makes it difficult to establish translatability for restoration practitioners.

SCIENCE OF CORAL STOCK SELECTION

Requisite in any program of selective propagation is the identification of individuals or populations with the desired phenotype. Heat-tolerance phenotypes may be derived from host, algal symbiont, microbial, or synergistic holobiont effects (see above) or inferred from the environment, and should be durable across space and time to be useful to practitioners. Strategies for identifying candidate coral colonies (**Table 1**) range dramatically in scalability, cost, lag time between conceptualization and usability, and technical dependencies.

There is evidence that each of these strategies has potential to identify a more heat-tolerant stock of corals for propagation than random or opportunistic sampling; however, there are pros, cons, and major unknowns for each. We assume that *in situ* methods will give more ecologically relevant information, but may not be as scalable or tractable as *ex situ* methods. While tank-based heat stress tests and molecular assays may be beneficial to research groups or small-scale projects where the investment in screening and tracking individual colonies is acceptable, more scalable solutions are critical for human interventions to have positive impacts on the long-term persistence of reefs. For example, opportunistic selection of coral stock from an area with documented elevated thermal history and/or an above average proportion of non-bleaching corals could be carried out with minimal additions to standard propagation workflows, but may suffer from lower precision. While more technically involved, remote sensing of individual corals across an entire reef potentially combines rapid identification with targeted selection. The most effective selection strategy for a given restoration project will depend on many factors including feasibility (resources, expertise), what prior data is available about the coral populations and the source and outplant sites, which particular coral species are included in the project, and the project scale and timeline.

TRADE-OFFS IN PROACTIVE CORAL REEF RESTORATION

The targeted selection of corals using any of these techniques should be expected to come with trade-offs because there

TABLE 1 | Approaches to identifying heat-tolerant coral stock.

Selection strategy	Summary	Limitations	Advantages	References
Local conditions	Local adaptation to environmental conditions increases likelihood of phenotype of interest	Not individual-based (probabilistic), does not account for plasticity, requires exploratory research, limited ability to capture full range of diversity	Logistically simple once established, inexpensive, *in situ*	Palumbi et al., 2014; Howells et al., 2016; Kenkel and Matz, 2016; Jury and Toonen, 2019; Schoepf et al., 2019; Quigley et al., 2020b; Voolstra et al., 2020
Known performance	Observed past performance of a colony is predictive of future performance	Need pre-established individuals monitored over time	Reliable, *in situ*, inexpensive, can be integrated into nursery propagation	Drury et al., 2017a; Fisch et al., 2019; Barott et al., 2020*; Matsuda et al., 2020; Ritson-Williams and Gates, 2020; Drury and Lirman, 2021
Stress tests	A sample representing the source colony undergoes heat stress, which is predictive of future performance	Not fully representative of natural performance, *ex situ*, requires aquaria infrastructure, limited scalability	Fast, reproducible, inexpensive once established	Barshis et al., 2013; Palumbi et al., 2014; Thomas et al., 2018; Morikawa and Palumbi, 2019; Voolstra et al., 2020
Host genetics	Using adaptive variants, epigenetics, or gene expression profiles to predict performance	Requires molecular work, expensive, high technical dependencies, unlikely to be single large-effect genes, may be species-specific	Mechanistic, targeted (within species), scalable, reproducible	Bay and Palumbi, 2014; Dixon et al., 2015; Rose et al., 2015; Kirk et al., 2018; Fuller et al., 2020; Quigley et al., 2020a; Drury and Lirman, 2021
Algal symbiosis	Algal symbiont communities influence holobiont performance	Requires molecular work, may be transient, some species do not harbor diverse assemblages	Potentially scalable, well-studied system, predictable tradeoffs	Rowan, 2004; Berkelmans and van Oppen, 2006; Sampayo et al., 2008; Cantin et al., 2009; Cunning et al., 2016
Biomarkers	Data correlated with performance (e.g., color, spectroscopy, metabolomics, lipidomics, proteomics, antioxidant activity) collected from novel individuals	May require molecular/bench work, low reproducibility, heavy developmental investment	Potentially scalable, fast with appropriate resources	Barshis et al., 2010; Innis et al., 2018; Mayfield et al., 2018; Majerova et al., 2020*; Williams et al., 2020*; Majerova and Drury, 2021*; Roach et al., 2021

**Indicates pre-prints available online.*

is an ultimate energetic budget allocated across the various metabolic, reproductive, and stress response processes in any organism (Lesser, 2013). Corals are exposed to multiple stressors including high temperatures, acidifying oceans, disease, salinity fluctuations, sediment, nutrients, algal overgrowth, and recurrent storms. It is still unclear whether building coral resilience to one stressor will in turn lead to resistance to multiple stressors ("cross-tolerance"; van Oppen et al., 2017). Previous work shows that growth in benign conditions was lower for stress-tolerant corals (Bay et al., 2017) and faster growth was inversely related to tissue loss after thermal stress (Ladd et al., 2017). High temperature tolerance was also inversely related to low temperature tolerance in transplanted corals (Howells et al., 2013) and migrants from warm climates suffered health consequences during winter (Schoepf et al., 2019). There is also strong experimental support for slower growth in heat-tolerant symbiont communities (Little et al., 2004; Jones and Berkelmans, 2010), likely the result of lower carbon translocation (Cantin et al., 2009), but this effect is diminished under warmer conditions like those corals will face in the future (Cunning et al., 2015a).

Muller et al. (2018) found no relationship between temperature tolerance and disease susceptibility in Caribbean Acroporids at ambient temperatures, but showed disease tolerance was lost under thermal stress, suggesting that a small proportion of the population is tolerant to both stressors. Conversely, Wright et al. (2019) found support for positive responses to multiple stressors. This study showed high correlations between temperature tolerance, calcification under ocean acidification conditions, and disease resistance, suggesting a possible common genetic architecture that could respond positively to selection, providing a mechanism for persistence under multiple stressful conditions (Wright et al., 2019).

At the population level, a potential cost of artificially accelerating local adaptation is reduced genetic or genotypic diversity. Selective propagation does not remove genotypes or standing genetic diversity from a reef (unlike agricultural monoculture), where relatively small numbers of coral genotypes (on the order of dozens) used as focal stock can capture nearly all the genetic diversity of a population (Drury et al., 2017b; Baums et al., 2019). However, selective propagation would shift the allele frequency spectrum, positively affecting patterns of directional selection during heat stress (Bay et al., 2017). Because heat-tolerance is a complex trait (i.e., there is more than one way for a coral to be heat-tolerant), rather than focusing on a single or small number of selected stock genotypes, restoration practitioners can choose to select as wide an assortment of corals as possible that meet the heat-tolerance selection criteria established by an individual project (Baums et al., 2019). This step will also likely result in individuals with multiple interacting pathways and genetic architectures that contribute to heat tolerance. To promote additional genetic diversity, restoration projects may leave substrate available for natural recruitment and emphasize coral survival and reproductive competence to maintain gene flow with other populations.

The evaluation of trade-offs in coral resilience is challenging because of the difficulty in extensive measurement of the many realistic phenotypes of interest, such as partial mortality, wound healing, growth rate, and fecundity (Baums et al., 2019). Changes at ecosystem scale may also be decoupled from experimental trade-offs, such that outcomes defined in one or several genotypes or species obscure broader functional dynamics on actual reefs. Regardless, delaying new interventions because of uncertainty around trade-offs could mean losing key species and functions (Anthony et al., 2020), representing an opportunity cost of non-intervention. A robust coral reef ecosystem is dependent on many coral traits, but we contend that temperature tolerance is of paramount importance in the face of ever-increasing coral bleaching events. While greater fecundity, growth, and structural complexity enhance ecosystem services and long-term capacity for resilience, corals that do not survive cannot contribute at all.

CONCLUSION

We argue that selection and propagation of heat-tolerant coral stock is a rational option for proactive reef restoration under climate change. We acknowledge risks and unknowns that warrant attention and further exploration, but contend that given the urgency of the situation, this strategy is feasible, relatively conservative, and logistically practical within existing restoration frameworks. Important areas for continued research include developing high-throughput selection methods, investigating the trade-offs in selecting heat-tolerant corals, and assessing the long-term viability of those corals. We also advocate empirical field tests to develop methodology, reveal unknown limitations and drawbacks, and assess real-world performance in preparation for implementing full-scale proactive restoration projects to meet resource management objectives and prepare coral reefs to face the future.

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

CC, KH, and CD conceived of and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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METHODS
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An Experimental Framework for Selectively Breeding Corals for Assisted Evolution

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Coral cover on tropical reefs has declined during the last three decades due to the combined effects of climate change, destructive fishing, pollution, and land use change. Drastic reductions in greenhouse gas emissions combined with effective coastal management and conservation strategies are essential to slow this decline. Innovative approaches, such as selective breeding for adaptive traits combined with large-scale sexual propagation, are being developed with the aim of pre-adapting reefs to increased ocean warming. However, there are still major gaps in our understanding of the technical and methodological constraints to producing corals for such restoration interventions. Here we propose a framework for selectively breeding corals and rearing them from eggs to 2.5-year old colonies using the coral *Acropora digitifera* as a model species. We present methods for choosing colonies for selective crossing, enhancing early survivorship in *ex situ* and *in situ* nurseries, and outplanting and monitoring colonies on natal reefs. We used a short-term (7-day) temperature stress assay to select parental colonies based on heat tolerance of excised branches. From six parental colonies, we produced 12 distinct crosses, and compared survivorship and growth of colonies transferred to *in situ* nurseries or outplanted to the reef at different ages. We demonstrate that selectively breeding and rearing coral colonies is technically feasible at small scales and could be upscaled as part of restorative assisted evolution initiatives. Nonetheless, there are still challenges to overcome before selective breeding can be implemented as a viable conservation tool, especially at the post-settlement and outplanting phases. Although interdisciplinary approaches will be needed to overcome many of the challenges identified in this study, selective breeding has the potential to be a viable tool within a reef managers toolbox to support the persistence of selected reefs in the face of climate change.

Keywords: selective breeding, larval rearing, nursery, restorative efforts, outplanting, growth, monitoring, survivorship

INTRODUCTION

The Anthropocene, the era in which humans have become a global geophysical force, is characterized by the degradation of ecosystem structure and function, loss of biodiversity and increased rates of species extinction (Steffen et al., 2007; Ceballos et al., 2015). Unfortunately, many existing conservation practices that are based on local management are inadequate in the face of global scale stressors such as those caused by climate change (Lennon, 2015). Coral reefs are among the ecosystems most impacted by human activities and climate change (Hoegh-Guldberg et al., 2019), leading to more rapid increases in extinction risk for many coral species compared to mammals, birds, and amphibians (Bongaarts, 2019). During the last 30 years coral cover worldwide has decreased by an estimated 20% (Hoegh-Guldberg et al., 2019), and four pan-tropical coral bleaching events since 1983 have led to coral declines on hundreds of reefs (Lough et al., 2018). Catastrophic coral bleaching and mortality driven by high sea temperatures occurred throughout Australia's Great Barrier Reef Marine Park, between 2015 and 2017, highlighting the limitations of localized management of reef fisheries and water quality (Hughes et al., 2017). The present rate of reef degradation emphasizes the urgent need to develop innovative conservation approaches that can maintain ecosystem services and ecological function despite projected sea warming owing to climate change.

As a result of anthropogenic climate change, the frequency, duration, and intensity of marine heat waves increased more than 20-fold between 1981 to 2017 (Laufkötter et al., 2020). Global mean sea surface temperature is projected to reach 1.5°C above that in pre-industrial times between 2030 and 2052, suggesting that shallow water corals have ∼10–30 years to adapt to this temperature increase (Hoegh-Guldberg et al., 2019). For many coral species this period will be too short for adaptation to happen by natural selection, given the sporadic nature of heatwaves at local scales (Bay et al., 2017). Even if warming can be limited to <1.5°C, it is highly likely that large areas will be experiencing regular mass bleaching events, threatening 70–90% of reefs by 2050 (Hoegh-Guldberg et al., 2018). In addition to tackling climate change, traditional conservation efforts will likely need to be coupled with restoration to assist recovery from disturbances (Anthony et al., 2017). Innovative solutions for actively assisting coral populations to pre-adapt to climate change *via* assisted evolution have been proposed to be included in management strategies for coral reefs (van Oppen et al., 2015). The goal of assisted evolution is to deliberately enhance certain traits in selected organisms, increasing their chances of surviving in the face of global change (Jones and Monaco, 2009). Such practices may involve induced acclimatization, modification of the microbial or the Symbiodiniaceae symbiont communities, and selective breeding (SB) for adaptive traits.

Selective breeding is the process by which humans choose individuals with specific heritable phenotypic traits to breed together and produce offspring. Humans have practiced SB for centuries to improve the production and taste of crops and livestock (Denison et al., 2003). More recently, such practices have been used to select for traits that might be beneficial in a changing climate such as drought resistance in plants (Hu and Xiong, 2014). SB can also be used as a conservation method for preserving populations of endangered species, however, there are only a few examples where this has been considered as a management strategy (Jones et al., 2007; Aitken and Bemmels, 2016). In marine invertebrates, SB has been used primarily in mollusk aquaculture to improve their growth (Hollenbeck and Johnston, 2018), protein content (Gjedrem et al., 2012), and disease resistance (Parker et al., 2012), highlighting that this approach can be adapted for calcifying organisms. To successfully conduct SB, adult colonies with adaptive, heritable traits (i.e., heat tolerance, growth rate, reproductive output, etc.) need to be selected as broodstock. Selecting for heat tolerance in corals is one of the approaches proposed in assisted evolution initiatives, given the importance of this trait in climate adaptation. There is evidence for heritability of heat tolerance at different life stages in some coral species under laboratory conditions (Csaszar et al., 2010; Dixon et al., 2015; Kenkel et al., 2015; Kirk et al., 2018; Quigley et al., 2020; Yetsko et al., 2020), suggesting that selective crosses between colonies with known tolerances could produce offspring with above-average heat resistance. Broodstock colonies can come from different populations exposed to contrasting temperatures profiles at a range of spatial scales (Dixon et al., 2015; Liew et al., 2020; McClanahan et al., 2020), or among individuals from within a single population where there is sufficient intrapopulation variability. While it is well established that coral populations experiencing higher mean sea surface temperatures or more variable temperatures tend to be more tolerant to heat stress (Howells et al., 2012; Thomas et al., 2018), less is known about the extent of within-population variation in heat tolerance (Bay and Palumbi, 2014; van Oppen et al., 2018). However, if sufficient variability does exist, then this approach has the advantage of reducing the likelihood of maladaptation to environmental variables other than temperature (Cotto et al., 2019) and may reduce the risk of inadvertently selecting different genetic variants or sub-species (however see Gomez-Corrales and Prada, 2020).

For assisted evolution methods to be successfully incorporated into resilience adaptation programs, they will need to be combined with techniques to restore and rehabilitate coral reefs (van Oppen et al., 2017). Some of the techniques associated with assisted evolution will rely on successful coral larval propagation (CLP) *via* sexual reproduction. For the purpose of this article, we define CLP as the process of producing and rearing corals from eggs through to colonies that are recruited into the population. We define a recruited colony as one that has been transplanted to the reef, has self-attached (sensu Guest et al., 2011), and contributes to the emergent properties of the population (growth, survivorship, and/or reproduction rates). CLP is an emerging method for producing large numbers of corals for reef rehabilitation and restoration, that overcomes early survivorship bottlenecks *via* a combination of land (*ex situ*) or ocean (*in situ*) based nurseries for rearing the early life stages. Several advances

have been made to improve the practices associated with CLP in recent years. The modes of sexual reproduction for approximately half of extant species of hermatypic scleractinians have been identified (Baird et al., 2009) and the timing of spawning cataloged for >300 Indo-Pacific coral species (Baird et al., 2021). CLP has been successfully executed in different geographical regions with several species under *ex situ* and *in situ* conditions (Omori, 2019; Randall et al., 2020) with sexually propagated colonies that were outplanted to the reef reaching sexual maturity (Nakamura et al., 2011; Baria et al., 2012; Guest et al., 2014; Chamberland et al., 2016). Competent coral larvae have been seeded *en masse* onto natural substrates to enhance recruitment (dela Cruz and Harrison, 2017; Doropoulos et al., 2019) and substrates have been designed to settle coral larvae for nursery rearing and outplantation improving early survivorship (Guest et al., 2014; Chamberland et al., 2017). Despite advances in the practice of CLP, most research has focused on the steps during and post-spawning, with little attention given to the provenance or phenotype of the broodstock colonies (apart from laboratory based studies, e.g., Dixon et al., 2015; Liew et al., 2020; Quigley et al., 2020). To the best of our knowledge, there have been no attempts to select parents for adaptive traits, such as heat tolerance, as part of CLP for reef rehabilitation.

For SB to be successfully implemented as a coral reef management tool we need to understand the biology of the trait of interest (**Figure 1A**) and also know how to logistically perform CLP using an assisted evolution approach (**Figure 1B**). Here we present a practical framework for developing, testing and implementing SB that can be adapted for coral reef rehabilitation and assisted evolution programs. In this study we combine SB trials with CLP using *Acropora digitifera* as a model species to select for heat tolerance. This framework can also be applied to other propagule-producing organisms (Vanderklift et al., 2020), and other adaptive traits like growth rate, disease resistance or wound healing capability (Baums et al., 2019). The proposed framework is structured into six sections: (1) selection of parental colonies with traits of interest based on phenotypic or other functional characteristics (e.g., known genotypic markers), (2) design of crosses for SB, (3) methods for collecting gametes to perform SB with corals, (4) methods of larval rearing and settlement onto substrate units *en masse* to produce coral colonies, (5) rearing of coral colonies (*in situ* or *ex situ*) for later outplanting to natural reef habitats, and (6) outplant of corals to the reef and monitoring of their growth and survivorship. Testing for heritability and potential resource trade-offs are also critical steps in SB (Ortiz et al., 2013; Cunning et al., 2015), and are being carried out as part of our ongoing work, however, the results of these studies will be reported elsewhere. For this study, we assume that heat tolerance is a heritable trait and that resource trade-offs between heat tolerance and other adaptive traits can maintain populations under future climate change scenarios. Both, the heritability of the trait and potential trade-offs are determinant for SB to be successfully implemented in the field. Further research needs to be done to confirm these assumptions, but these are outside the scope of the present study.

MATERIALS AND METHODS

Selection of Parental Colonies for Selective Breeding

The reef-building coral *A. digitifera*, was used as a model for SB as it is widely distributed and abundant on shallow reefs throughout the Indo-West Pacific. Its digitate morphology facilitates fragment removal for conducting stress assays, and spawning times are established for many locations (Keith et al., 2016; Baird et al., 2021). All of the work described here was carried out at the Palau International Coral Reef Center (PICRC) in the Republic of Palau located in Western Pacific Ocean (**Supplementary Material 1A**). The source site for all colonies is a shallow, exposed patch-like reef (Mascherchur, N 07°17′29.3″; E 134°31′8.00″; **Supplementary Material 1B**), where *A. digitifera* is abundant at depths ranging between 0 and 4 m. In November 2017, 99 visibly healthy adult coral colonies were tagged and mapped along eleven 20-m long fixed transects. The distance between the selected colonies was at least 3 m to maximize the chance of sampling distinct genets rather than clonemates. From these 99 colonies, 34 were randomly selected to assess their performance during a short-term (7-day) temperature stress assay to select parental colonies for the broodstock.

For this short-term assay, seven ∼3 cm long fragments were excised from each colony and transported by boat in 50 L seawater tanks to PICRC (∼20 min boat travel time). The donor colonies remained on the reef to recover for approximately five months before SB work began. The 238 fragments were glued to aragonite substrata (∼20 mm diameter, Oceans Wonders LLC) with ethyl cyanoacrylate gel (Coraffix gel), labeled and mounted into plastic holders, that were attached to plexiglass racks (**Supplementary Material 2A–J**). To determine the relative heat tolerance of each colony, a 7-day temperature stress experiment was performed using two temperature levels: (a) ambient seawater temperature conditions (30.37 ± 0.46°C, three replicate tanks, **Supplementary Material 2C,F,I**), and (b) heat stress conditions (**Supplementary Material 2A,B,D,E,G,H**), where temperature was raised incrementally over the course of 3 days (+2°C on day 1, and +1.5°C on day 3), reaching a daily average temperature of 32.95°C (±0.37) during days 4–7 (five replicate tanks, **Supplementary Material 3**). Replicate fragments were randomly distributed among seven treatment tanks (24 fragments per tank), with all colonies having at least two replicates in independent stress tanks and at least one replicate in an ambient temperature tank (used as a control for handling stress). The status of each fragment was visually inspected by the same observer daily and ranked as: (1) healthy (no signs of discoloration or mortality), (2) partial mortality (less than 30% of surface area dead) or, (3) dead (more than 30% of the surface with bare skeleton and without tissue). Relative heat tolerance was determined by the end-point mortality (6 days after the first temperature increase). Colonies with all replicate stressed fragments alive (0% mortality) were considered to have

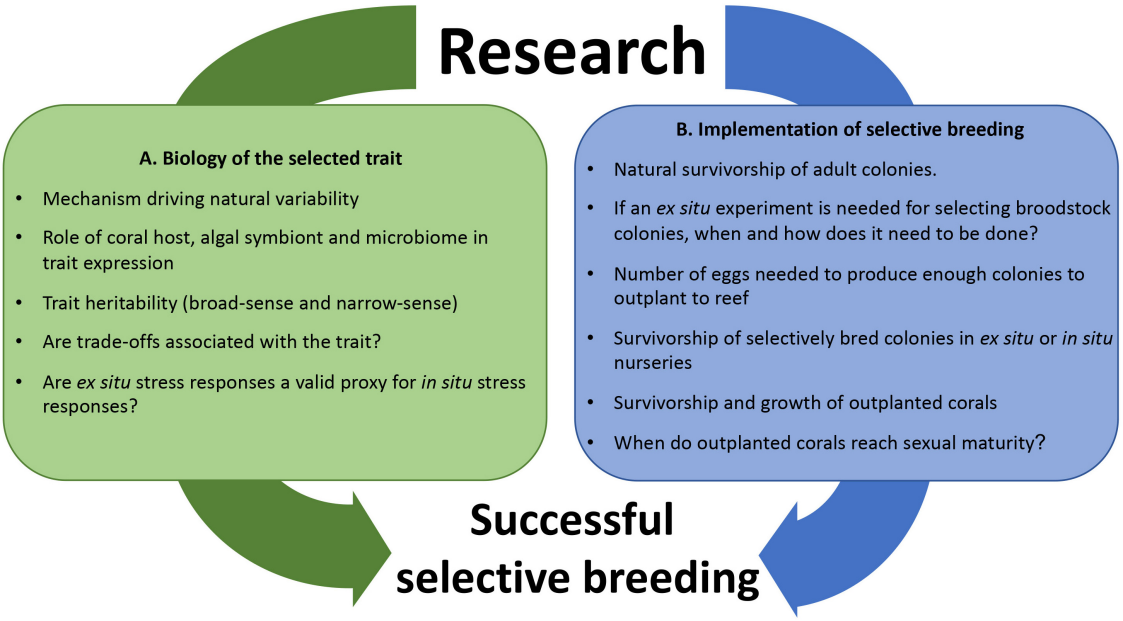

FIGURE 1 | Illustration of research needs for coral selective breeding related to **(A)** biology of the selected trait, and **(B)** implementation in the field. It is imperative to develop both research areas simultaneously as the time left to implement these conservation initiatives, given current climate change predictions, is very short (20–30 years).

relatively high heat tolerance (RHHT), whereas colonies with all stressed fragments dead (100% mortality) were classified as having relatively low heat tolerance (RLHT). Colonies that were not classified either as RHHT or RLHT were considered as unclassified. For brevity and ease of comprehension we will henceforward usually refer to RHHT colonies as "highs" and RLHT as "lows" in the main text but one should be clear that the terms are purely relative and pertain to the particular stress test conducted. Relative heat tolerance was considered as unresolved for colonies with control fragments held at ambient temperature that showed a stress response (partial mortality or death), as this might have resulted from handling stress. Relevant National and State permits were obtained for the collection of fragments (National Marine Research Permits: RE-018, RE-18-13).

Coral Spawning

In anticipation of *A. digitifera* spawning in Palau (Penland et al., 2003; Gouezo et al., 2020), the 34 colonies used in the temperature stress assay were surveyed to assess reproductive (gravid or non-gravid) and health (alive, partial mortality, or dead) statuses before the April full moon (April 1, 2018). Reproductive status was established by fracturing two branches per colony and checking for the presence of visible pigmented oocytes (**Figure 2A**; following Baird et al., 2002). Of the 12 colonies identified as lows and four colonies identified as highs (see sections "Materials and Methods" and ""Results"), five and three colonies respectively contained visible, pigmented gametes (**Supplementary Material 4**). Three gravid colonies were haphazardly chosen from each relative heat tolerance category and collected on March 29 for the SB crosses and transported in 50 L containers to PICRC. Colonies were maintained in an outdoor flow-through 760 L holding tank where water was mixed using three magnetic pumps (Pondmaster 1200 GPH). Four days after the full moon, setting (gamete bundles visible within polyp mouths) was observed in all colonies. We used standard coral larval rearing methods (Guest et al., 2010), with modifications to ensure that individual crosses were isolated. From sunset onward (19:00 h), colonies were checked visually for signs of bundle setting every 30 min. As soon as one colony was seen setting, all colonies were isolated in individual 80 L static tanks to prevent cross fertilization. When most bundles were released (**Figure 2B**), 200 ml plastic cups were used to scoop buoyant bundles from the water surface. Egg-sperm bundles were separated by transferring them onto a 100 μm mesh filter immersed in a bowl containing a small amount of UV-treated (Trop UV Sterilizer Type 6/IV – TPE, Trop-Electronic GMbH, Germany) 0.2 μm filtered sea water (FSW). Sperm remained in the bowl while eggs remained immersed in FSW within the filter. The filter was removed quickly and transferred to a new bowl with UV-treated 0.2 μm FSW, and eggs were washed five times to remove any sperm residue. Throughout this process, all bowls, filters and other utensils were rinsed with diluted bleach (1%) and FSW. All implements were labeled and used exclusively for individual colonies or crosses to avoid cross contamination. After spawning, colonies were returned to the holding tank, and a week later they were transplanted at the natal reef (Mascherchur).

Fertilization and Selective Crosses

Separated gametes were cross fertilized to produce two types of crosses (1) high sire × high dam, and (2) low sire × low

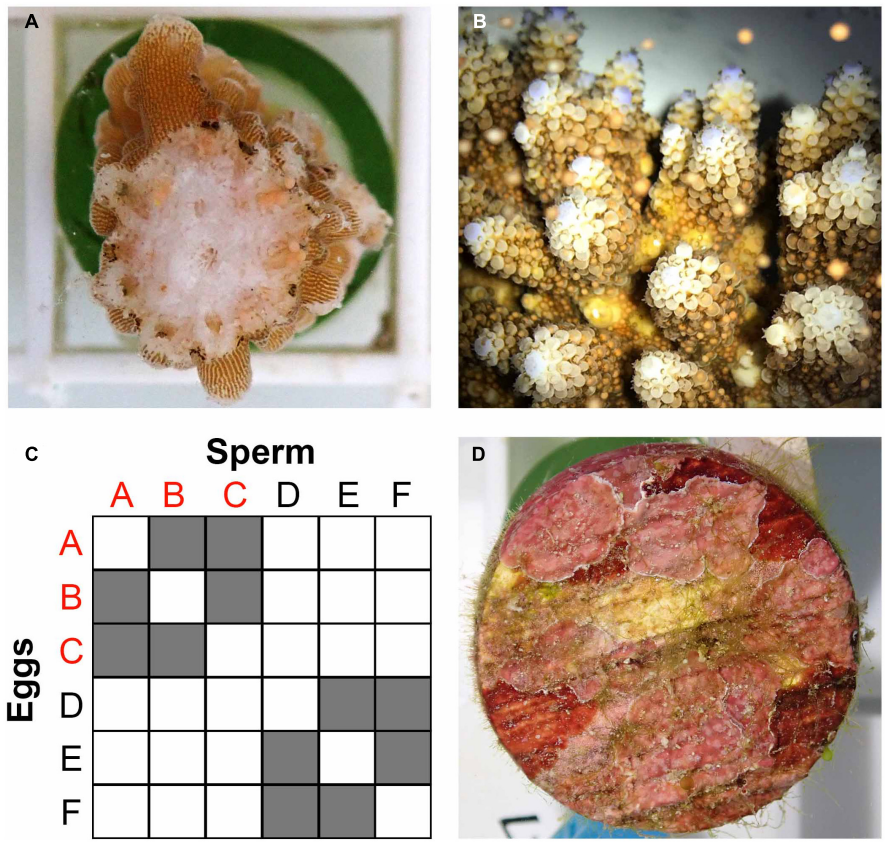

FIGURE 2 | (A) Fragment of a gravid colony of *Acropora digitifera* with pigmented eggs. **(B)** *A. digitifera* bundle release. **(C)** Schematic representation of the 12 selective breeding crosses performed using three parental colonies of *A. digitifera* with relatively high heat tolerance (RHHT, colonies A, B, and C), and three colonies with relatively low heat tolerance (RLHT, colonies D, E, and F). **(D)** Substrate unit conditioned with CCA before been offered to larvae for settlement.

dam, and each type of cross was replicated six times using different combinations of parental colonies to produce 12 unique crosses (**Figure 2C**). The collection and separation of gametes, the performance of SB crosses, the washing of embryos after fertilization, and the maintenance of cultures were carried out by six researchers, two of them with expertise in *ex situ* coral spawning (JRG and AH). The resulting crosses were maintained in 15 L cone-shaped tanks (Pentair Vaki Scotland Ltd.) at ambient temperature, with 0.2 L/min flow-through with UV-treated 0.2 μm FSW, resulting in one turnover per hour per tank (**Figure 3**, **Supplementary Material 5**). A PVC "banjo" with a wedge shape, covered with 100 μm mesh filter was fixed to the inside of the outflows of the tanks to avoid loss of larvae. Each cross was divided between two rearing tanks, resulting in 24 larvae culture tanks. Gentle aeration was introduced 24 h after fertilization, when embryo development had progressed sufficiently, and larvae were round and motile.

Larval Settlement

Circular ceramic substrates (Oceans Wonders LLC) ∼2 cm in diameter with a 1.5 cm stem (hereafter referred as substrate units "SUs"), overgrown with crustose coralline algae (CCA; **Figure 2D**) were offered to the larvae for settlement. The SUs had been biologically conditioned for four months (130 days) before spawning in two 300 L holding tanks with flow-through water mixed using four pumps each (Taam Rio +800 Powerhead). SUs were arranged on plastic egg crates raised from the bottom of the tank, with fragments of CCA collected at Mascherchur placed on top of the SUs. To stimulate growth of CCA over the SUs and avoid the colonization of filamentous algae, frames with shading cloth were placed over the tanks to reduce light levels to 4 μmol photons m^{-2} s^{-1}. Three days after fertilization, larvae were transferred from 24 larval rearing tanks to 24 settlement tanks filled up with 10 L of 10 μm FSW and 80 conditioned SUs each. Half of the water in each tank was changed daily with new FSW. Two days after larvae were moved to the settlement tanks, SUs were transferred to flow-through nursery tanks (*ex situ* nurseries). Each SU was tagged with a cable tie using a color coded system to identify from which cross and replicate culture the colonies had originated (resulting in 24 color codes from 12 crosses).

Ex situ Nursery Tanks

Substrate units with settled corals were randomly distributed among four *ex situ* flow-through nurseries consisting of 184 L tanks (length: 128 cm, width: 85 cm, water level: 17 cm) with

FIGURE 3 | (A) Overview of the larval rearing system consisting of 15 L cone shaped tanks with flow-through. Sea water is filtered through four filters (50, 10, 5, and 0.2 μm) and then exposed to UV light before entering into the tanks. Each tank has inflow pipe for the filtered and UV treated sea water, an outflow pipe for waste-water, and an airline connected to an air pump. **(B)** Diagram showing the main components of the larval rearing system. Arrows indicate the direction of water flow. (a) Unfiltered sea water inflow, (b) polyvinyl chloride (PVC) valve 1", (c) 1" PVC pipe, (d) first filtering station (50 μm), e) 3/4" pipe, (f) Second filtering station (10, 5, 1, or 0.2 μm), (g) 1/2" pipe, (h) Trop UV Sterilizer, (i) PVC valve 1/2", (j) 15 L cone-shaped tanks, (k) Banjo with 100 μm mesh filter, (l) Water inflow tube 1/4", (m) Elbow 1/4", (n) Valve 1/4", (o) Water outflow tube 1/2", (p) Air pump, (q) Airline, and (r) 1" PVC waste pipe.

50 μm FSW. Each tank was illuminated with two Aquarium lights (48" 50/50 XHO Led, Reef Brite Ltd.) at an intensity of 200 μmol photons m^{-2} s^{-1} over a 12:12 h diurnal cycle, and had two pumps (Hydor Koralia Nano Circulation Pump/Powerhead, **Figures 4A,B**) to create water circulation. Fragments from each parental colony were added to each tank to promote Symbiodiniaceae uptake by the coral settlers, together with fragments of CCA. To minimize growth of turf algae, eight small herbivorous juvenile rabbitfish (*Siganus lineatus*,∼5 cm long) and numerous small grazing snails

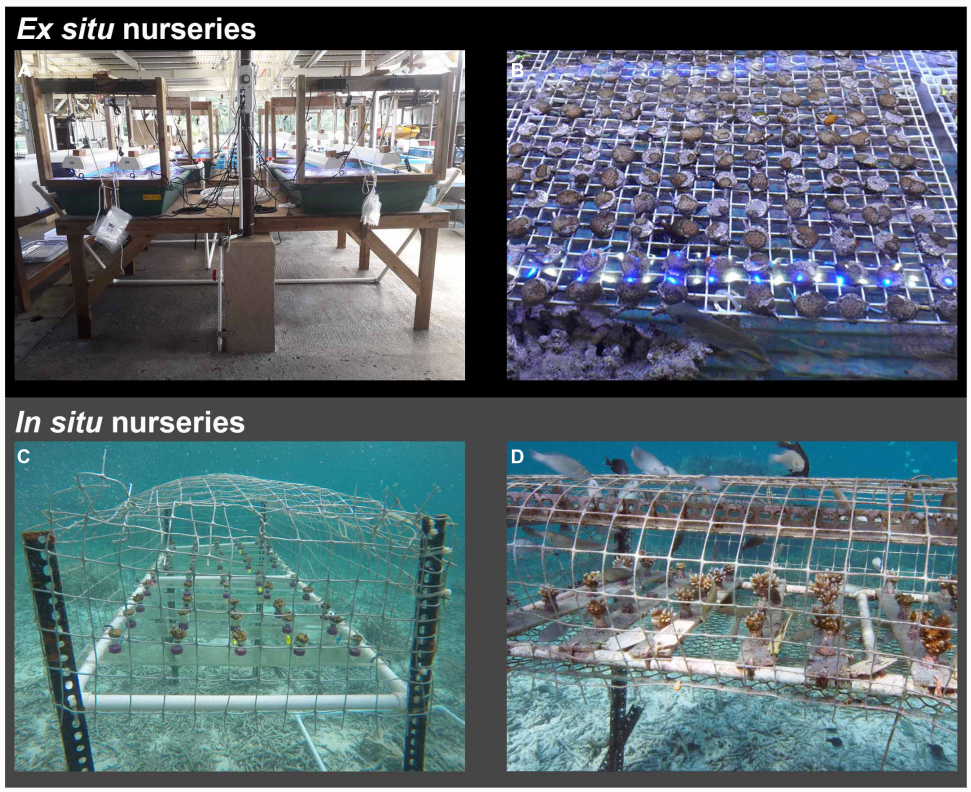

FIGURE 4 | (A) *Ex situ* nursery tanks, **(B)** detail of *ex situ* nurseries with the presence of herbivores *Siganus lineatus*. **(C)** Coral colonies when transferred from the *ex situ* to the *in situ* caged nursery (13-months old). **(D)** Corals (17-months old) in the *in situ* nursery showing the presence of small herbivore fish within the cage.

(*Cerithium* sp.) were added to each tank. Fish were fed daily with fish pellets (Ocean Nutrition Formula) and nurseries were cleaned every other week by siphoning off the detritus from the bottom of the tanks.

Coral Outplanting to the Reef

Corals from 12 crosses were outplanted from the *ex situ* nursery to the natal reef of parental broodstock (Mascherchur) when colonies were 5- and 11-months old (144 and 318 days, $n = 288$ and 96 colonies respectively, **Supplementary Material 6**). The number of colonies outplanted at 11-months was limited by the workforce available at the time. To facilitate monitoring, SUs with corals were outplanted along 16 fixed 10-m transects at a depth interval of 1.5–4 m. To attach each SU to the reef substrate, 11 mm holes were drilled into bare reef substratum with a submersible cordless drill (Nemo Divers Drill) and SUs were glued with epoxy (Milliput Standard) after cleaning the reef surface area with a wire brush. Nails were hammered next to each SU to which a cable tie, color coded for each cross was attached. Two divers were required to outplant 25 corals in one 2-h dive. Colonies were monitored at 11, 17, 25, and 32-months (318, 515, 767, and 974-days old respectively) to assess their status (alive, missing, or dead) and photographed from directly above with an underwater camera (Olympus Tough TG-5), and with a ruler for scale.

In situ Nurseries

After 13-months (386-days) of *ex situ* rearing, the remaining colonies on 296 SUs were transferred to six *in situ* nurseries (N 7°18′19.80″N; E 134°30′6.70″E, **Figure 1B**) 2.20 km away from the natal reef. Nurseries were constructed *in situ* using steel slotted angle bars 40 × 40 mm (length: 135 cm, width: 60 cm), raised from the seafloor (85 cm). A plastic mesh (aperture size 5 cm) was used to cover the nursery structures (**Figures 4C,D**) to exclude larger corallivores (Baria et al., 2010). Corals were attached to plexiglass racks with five colonies per rack, spaced 3 cm apart. Ten racks were placed in each nursery resulting in a total of 50 colonies per nursery. Meshes and racks were cleaned monthly using a stiff plastic brush to remove algal overgrowth. When colonies were 17-months old (504 days), each colony was photographed with an underwater camera (Olympus Tough TG-5) from directly above, and with a ruler for scale.

Costs of Producing, Rearing, Outplanting, and Monitoring ∼2.5 years Old Coral Colonies Using a Selective Breeding Approach

The cost of producing ∼2.5-years old (32-months) live colonies was calculated from the total cost of materials and hours of labor needed to run the experimental setup at full capacity with 24

larval cultures, rear, outplant at the natal reef and monitor the resulting colonies. The cost of the experiment to characterize the relative heat tolerance of the parental colonies (**Supplementary Material 7**) was not included in this analysis, as this will vary considerably according to the trait of interest being selected for, the methodology (i.e., experiments under laboratory conditions, type of analysis, etc.) used to identify colonies of interest, and their location. Due to logistical constraints, we were not able to quantify fertilization success, larval survivorship, and initial settlement densities and survivorships, hence, values reported in the literature were used for the analysis. Details of the assumptions of the analysis, costs of consumables, equipment, and person hours are provided in the **Supplementary Material 7**. Cost per coral was estimated by dividing the total cost for the project by the number of SUs containing one surviving 2.5-year old coral for different ages at outplant (5 or 11-months). To compare costs of rearing in *ex situ* and *in situ* nurseries we considered the costs of consumables for their construction and their maintenance during a 11-month period, and cost per SUs was estimated by dividing the total cost of building and maintenance of the nurseries by the number of SUs. The overall efficiency for each outplantation age (5 or 11-months) was estimated by dividing the number of coral eggs used by the number of living colonies after 2.5-years. The aim of this analysis was to: (1) estimate the minimum cost of CLP using a SB framework, (2) identify the steps of the framework (coral collection, spawning to competency, settlement, rearing, outplanting, and monitoring) that incur the highest cost of the budget, and (3) evaluate the effect on efficiency and cost per coral of outplanting colonies at two ages (5 and 11-months). The total cost of this framework should not be used as a reference for SB under assisted evolution since: (1) the costs resulted from a small spatial scale experiment and are not representative of expenses of restoration initiatives at large spatial scales, (2) specific assumptions were made for its computation (**Supplementary Material 7**) and any change in these assumptions will change the total costs, (3) upscaling each of the steps will reduce their cost due to economies of scale, and (4) our results do not include data on the reproductive status of the recruited colonies 2.5 years post-fertilization. Before the predicted spawning event in 2020, the reproductive status of nine colonies with the biggest diameter in the *in situ* nursery was assessed (as described in section "Coral Spawning"), with none containing visible eggs. Additionally, recruited colonies had smaller sizes than the corals in the *in situ* nursery and were only just starting to develop branches (**Figure 5**), suggesting that they were not reproductive at this time.

Settlement Density

To provide practical guidance for planning CLP, the minimum number of settlers needed to obtain SUs with at least one colony after four-months (132-days old) of nursery rearing was determined by testing the effect of settlement density on colony survivorship. This experiment was carried out during the spawning event on April 6, 2020 using three adult *A. digitifera* colonies collected at Mascherchur. A mass culture was produced with the gametes of the colonies following the protocol previously described for collecting bundles and rearing larvae. Four days after spawning, once larvae were competent to settle, 1.5 L static tanks were stocked at three levels of larval densities (10, 25, and 50 larvae per SU or 67, 167, and 333 larvae L^{-1}, with $n = 8$ replicate tanks). Each tank contained between four and ten SUs previously conditioned with CCA for 198 days (**Supplementary Material 9**). Water changes were carried out twice daily over a week with UV treated 1 μm FSW. Ten days after settlement, the number of settlers on each SUs were counted using a stereomicroscope. SUs ($n = 157$) with live settlers (between one and 15 per SU) were then randomly distributed across four *ex situ* nurseries (described in section "*Ex situ* Nursery Tanks"). The number of live corals per SU was again recorded after 4 months using a stereomicroscope.

Data Analysis

Natural mortality of tagged colonies on the reef was estimated using yearly exponential rates of survival (Clark and Edwards, 1995). Colony survivorship was compared between the two different outplanting times to the reef (5 and 11-month old) using right censored data with the Kaplan–Meier model and the log-rank statistic (Harrington and Fleming, 1982). As it was not possible to determine the exact time of death for each coral, the date that a coral died was estimated as the middle time point between survey dates. Survivorship functions of corals were compared (a) among outplanting times at different ages (5 and 11-months old), and (b) once outplanted i.e., with respect to days out on the reef rather than age attained. Colony size, measured as planar area for corals outplanted to the reef (at an age of 5 and 11-months) or moved to the *in situ* nursery was estimated from scaled downward-facing images taken when corals were 17-months old using ImageJ. During image analysis, the number of developed branches per colony was recorded as an indicator of volume. The number of colonies with branches was compared using basic descriptive summary methods (percentages of branching colonies of the total alive outplants). The effect of outplanting method (three-level fixed effect) on colony size at 17-months was tested using Generalized Linear Mixed effects Models (GLMM; Brooks et al., 2017), accounting for differences in size due to cross replication (12-level random factor). Log-Gamma link was used to prevent negative fitted values of this strictly positive response variable. Among method comparisons were tested using a *post hoc* Tukey Test.

The effect of larval culture density (three-level fixed effect) on settlement density after 10 days was tested using GLMM, accounting for variability among settlement tanks (eight-level random effect). The relationship between settlement density (fixed effect) and number of 4-month-old colonies per SU was tested using GLMM, accounting for variability among holding tanks (four-level random effect). All model validation steps included assessing homogeneity of residuals versus fitted values, over and under dispersion and a simulation study to test the ability of the model to capture zero-inflation (Zuur and Ieno, 2017). Poisson models suitable for count data (Bates et al., 2015) were over-dispersed due to underestimation of zeros, however, negative binomial models with quadratic parameterization variance structure (Brooks et al., 2017) passed all validation routines (**Supplementary Material 9**). Marginal R^2

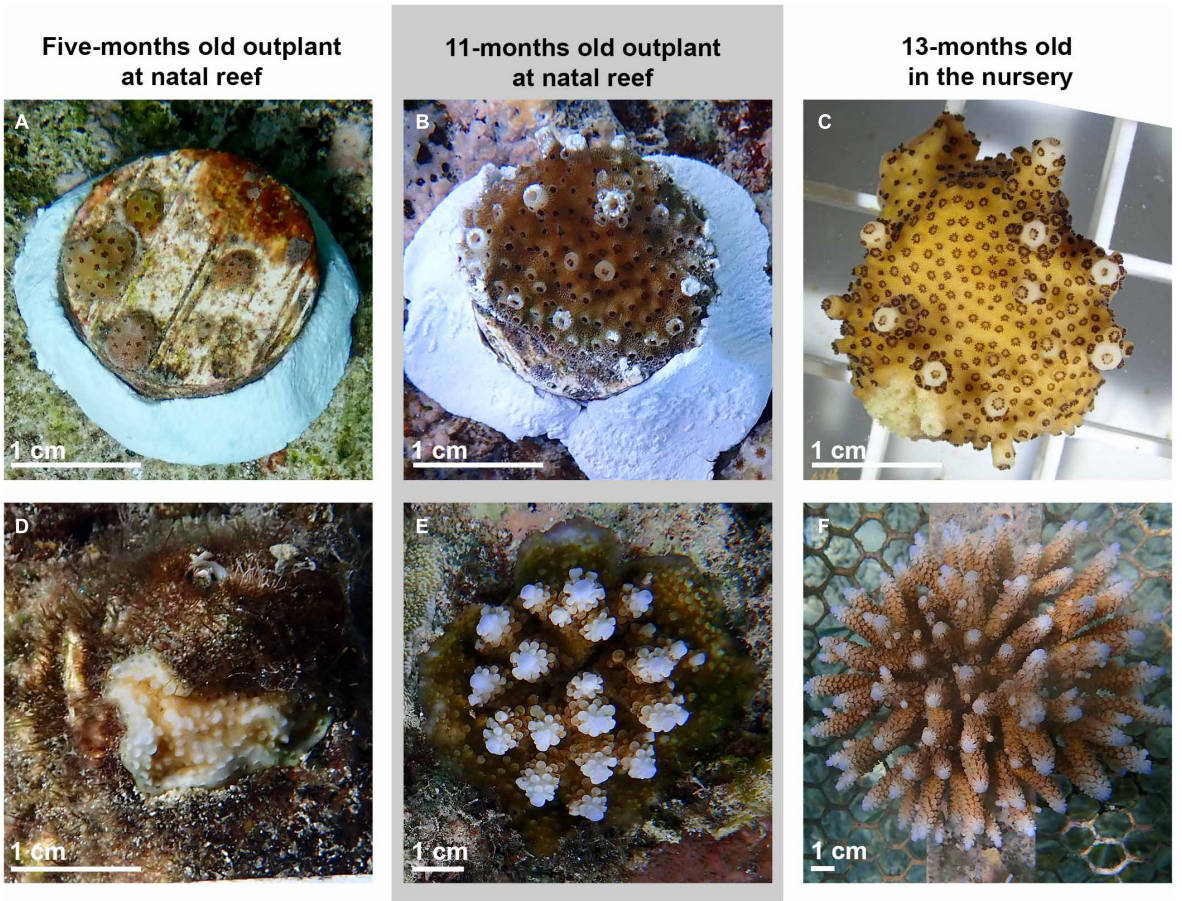

FIGURE 5 | Images showing representative colonies to illustrate differences in size and morphology at the time of outplanting to the reef, or transfer to the *in situ* nursery (**A**: 5-month old outplant, **B**: 11-month old outplant, and **C**: 13-month old before transferring to the *in situ* nursery), and during the last monitoring (**D**: 5-month old outplant, **E**: 11-month old outplant, and **F**: *in situ* nursery) when colonies were 2.5 years old (52 days after the spawning event of 2020).

values, the proportion of variance explained by fixed effects alone were computed for the final model (Lüdecke et al., 2020).

RESULTS

Selection of Parental Colonies

Relative heat tolerance varied among colonies (n = 34), with 15% categorized as high and 32% categorized as low (**Table 1**). For 21% of the colonies at least one of the fragments held in the control tanks died, and therefore their relative heat tolerance was considered as unresolved (**Table 1**). For the remaining 32% colonies, between 25 and 66% of fragments died during the experiment and colonies were unclassified in terms of relative heat tolerance (**Table 1**). Natural mortality rates of tagged colonies at Mascherchur were estimated at ∼20% per year, with eight colonies recorded as dead 140 days after tagging (n = 99). Partial mortality was observed in 6% of the tagged colonies and one colony could not be relocated (**Supplementary Material 4**). From the surviving, visibly healthy colonies (n = 84), 38% contained pigmented eggs in March 2018, and these included three colonies classified as high and five as low (**Supplementary Material 4**).

Settlement Density Experiment

In 2020, the density of the larval culture had a significant effect on settlement densities (**Figure 6A**). The expected mean settlement density using a larval culture of 50 larvae per SU was two- and threefold higher than that of the two other larval cultures with 25 and 10 larvae per SU respectively (GLMM, R^2 = 0.28, $p < 0.01$; **Supplementary Material 10**). The density of live settlers per SU at 10 days post-settlement had a positive effect on the density of colonies four months later (**Figure 6A**, GLMM, R^2 = 0.36, $p < 0.001$; **Supplementary Material 11**). On average, a settlement density of four settlers per SU was sufficient to obtain at least one coral per SU after four months under *ex situ* nursery conditions (1.3 settlers per cm^2 of effective settling surface, **Figure 6B**).

Effects of *ex situ* and *in situ* Nursery Rearing on Colony Survivorship

Colony survivorship was significantly affected by age at the time of outplanting ($F_{1,380}$ = 102, $p < 0.05$, **Figure 7A**). The median

TABLE 1 | Results of the 7-day heat stress exposure used to determine the relative heat tolerance of the colonies.

Relative heat tolerance	Stress treatment			Control treatment	
	# fragments	# fragments dead	% fragments dead	# fragments	# fragments dead
Low	2	2	100	1	0
Low	5	5	100	1	0
Low	5	5	100	1	0
Low	3	3	100	1	0
Low	2	2	100	3	0
Low	2	2	100	1	0
Low	4	4	100	1	0
Low	2	2	100	2	0
Low	3	3	100	2	0
Low	3	3	100	2	0
Low	3	3	100	2	0
Low	4	4	100	1	0
High	4	0	0	1	0
High	5	0	0	1	0
High	5	0	0	1	0
High	5	0	0	1	0
Unclassified	4	2	50	1	0
Unclassified	5	2	40	1	0
Unclassified	3	1	33	1	0
Unclassified	3	2	66	1	0
Unclassified	3	1	33	3	0
Unclassified	3	2	66	3	0
Unclassified	4	2	50	2	0
Unclassified	3	1	33	1	0
Unclassified	3	2	66	1	0
Unclassified	3	2	66	1	0
Unclassified	4	1	25	1	0
Unresolved	2	0	0	3	1
Unresolved	4	4	100	2	1
Unresolved	3	3	100	1	1
Unresolved	3	3	100	1	1
Unresolved	3	3	100	3	1
Unresolved	3	3	100	1	1
Unresolved	3	1	33	2	2

survival age of corals outplanted at 11-months old was more than twice that of those outplanted at 5-months old (median survival age of 646 and 257 days respectively). The relative survival once outplanted (i.e., with respect to days out on the reef) increased more than threefold when *ex situ* nursery time was extended, with colonies outplanted 5-months post-fertilization surviving a median time of 104 days, whereas outplants at 11-months post-fertilization survived for a median time of 324 days post-outplant. Only ∼6% of corals that were outplanted at 5-months post-fertilization survived to 32-months old. In contrast, corals that were reared in nurseries for 11-months prior to outplanting to the reef had five times better survivorship (∼30%) to 32-months old.

At 17-months, corals at the *in situ* nursery started developing a 3D structure with branches present in 72% of the colonies, compared to corals outplanted to the reef where none had started branching for both 5 and 11-months outplants. The planar area of corals was greater in treatments that had longer husbandry times (i.e., were held in the *in situ* or *ex situ* nurseries for longer periods). Coral size at 17-months was strongly affected by age at time of outplanting (i.e., outplanted to the reef at 5-month, 11-months, or transferred to the *in situ* nursery after 13-months, **Figures 7B,C**). *In situ* nursery reared corals were significantly larger than those outplanted at 5 and 11-months (GLMM $p < 0.001$, **Supplementary Material 12**), and corals outplanted at 11-months were significantly larger than those outplanted at 5-months (GLMM Tukey Test, $p < 0.001$).

Cost Analysis

The total cost of producing, rearing, outplanting, and monitoring 2.5-years old corals using a SB framework was US$23,817 for colonies outplanted at 5-months old (**Supplementary Material 13, 14**), and US$22,500 for those outplanted at 11-months old (**Table 2** and **Supplementary Material 15**). The total cost when outplanting corals at 11-months old was US$1,317 less than when

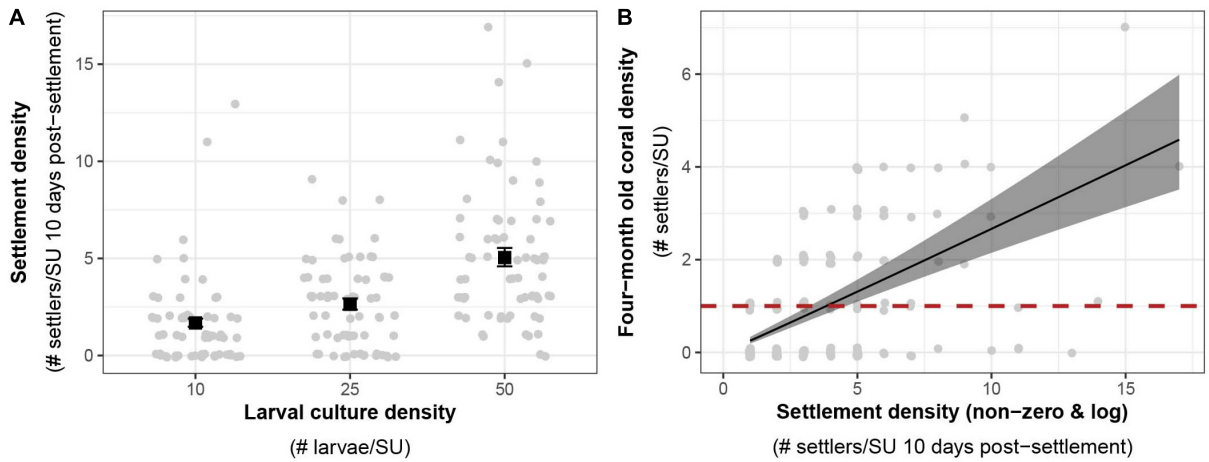

FIGURE 6 | (A) Effects of larval density on settlement density. Average expected response values among settlement tanks (black squares) from negative binomial GLMMs are shown with standard error bars. **(B)** Expected relationship between settlement density and colony density after 4 months (132 days, black line and standard error) among *ex situ* nursery tanks derived from a negative binomial GLMM. Equation: *four months coral density = exp[−1.44 + 1.02 × log(Non-zero settlement density)]*. The critical target threshold of 1 coral per substrate after four months is represented with a red dashed line. Raw count data (gray circles) are visualized with a jitter to minimize overlap of points in the two graphs.

outplanting at 5-months, because a greater number of colonies (300 extra colonies) needed to be outplanted and monitored thus incurring higher labor and consumable costs. The overall efficiency to produce 12 distinctive crosses with two replicate cultures each and rearing the resulting colonies under *ex situ* nursery conditions until outplant age (either 5 or 11-months old) was increased fourfold when outplanted at the later age (0.09 and 0.4, respectively). Equally, the cost of each SU with a live coral after 2.5 years was five times lower when nursery period was extended (US$227 for outplants at 5-months old compared to US$49 for 11-months old outplants). Outplanting was the activity that incurred the highest costs (35 and 32% of the total cost for the 5 and 11-months old respectively; **Table 2** and **Supplementary Material 15**), followed by monitoring (33 and 31% of the total cost for the 5 and 11-months old respectively, **Table 2** and **Supplementary Material 15**). Costs associated with rearing corals for 11-months in *ex situ* compared to *in situ* nurseries differed considerably, with cost per SUs being sixfold lower for the *ex situ* nurseries (US$0.97 and US$5.75 respectively, **Supplementary Material 15–17**).

DISCUSSION

Research to assess the feasibility of assisting adaptation of corals in the face of ocean warming is accelerating, however frameworks for practical application of this research to conservation and management are lacking. Here we present a framework for CLP that involves prior selection of parental colonies based on intrapopulation variation in their heat tolerance (**Figure 8**). We established optimal settlement densities of larvae to obtain one coral per SU after four-months of *ex situ* rearing and demonstrate the potential of rearing corals using a combination of *ex situ* and *in situ* nurseries to optimize their growth and survivorship. We found large differences in growth between corals outplanted to the reef and those reared in *in situ* nurseries. We also found differences in survivorship depending on age at outplant from *ex situ* nurseries, emphasizing the benefits of long nursery phases on final costs per coral. Our data highlight some of the major challenges associated with combining SB with CLP, and the areas that need further research and development to improve efficiency and to reduce the high costs involved.

The selection of parental colonies was made based on intrapopulation variation in heat tolerance using a 7-day temperature stress exposure (**Figure 8.1**). Our results show that our study population of *A. digitifera* contained considerable intrapopulation variability in heat tolerance, with some colonies (∼15%) able to withstand a short-term heat stress with zero fragment mortality. In this case, there was clearly sufficient intrapopulation variability in relative heat tolerance to provide broodstock for SB initiatives. While further work is needed to determine if the selected trait is passed on to offspring, the relative heat tolerance among the selected broodstock was not related to the Symbiodiniaceae community (**Supplementary Material 19**). The high annual mortality rate of the tagged colonies (9.5%) and the low proportion of the population spawning in the same month (38%) meant that we had relatively few colonies to choose from for SB. Rates of annual mortality recorded here are typical for *Acropora* (Madin et al., 2014) and spawning synchrony can vary considerably among taxa, location, and year (Baird et al., 2009; Gouezo et al., 2020), or can shift with handling and transportation stress or while in captivity (Okubo et al., 2006; Craggs et al., 2017). These facts highlight the need to identify and test relatively large numbers of corals within populations to increase the likelihood of having sufficient numbers of gravid colonies for SB. Selecting the broodstock from within populations (rather from distinct populations) avoids risks associated with maladaptation, genetic swamping or outbreeding

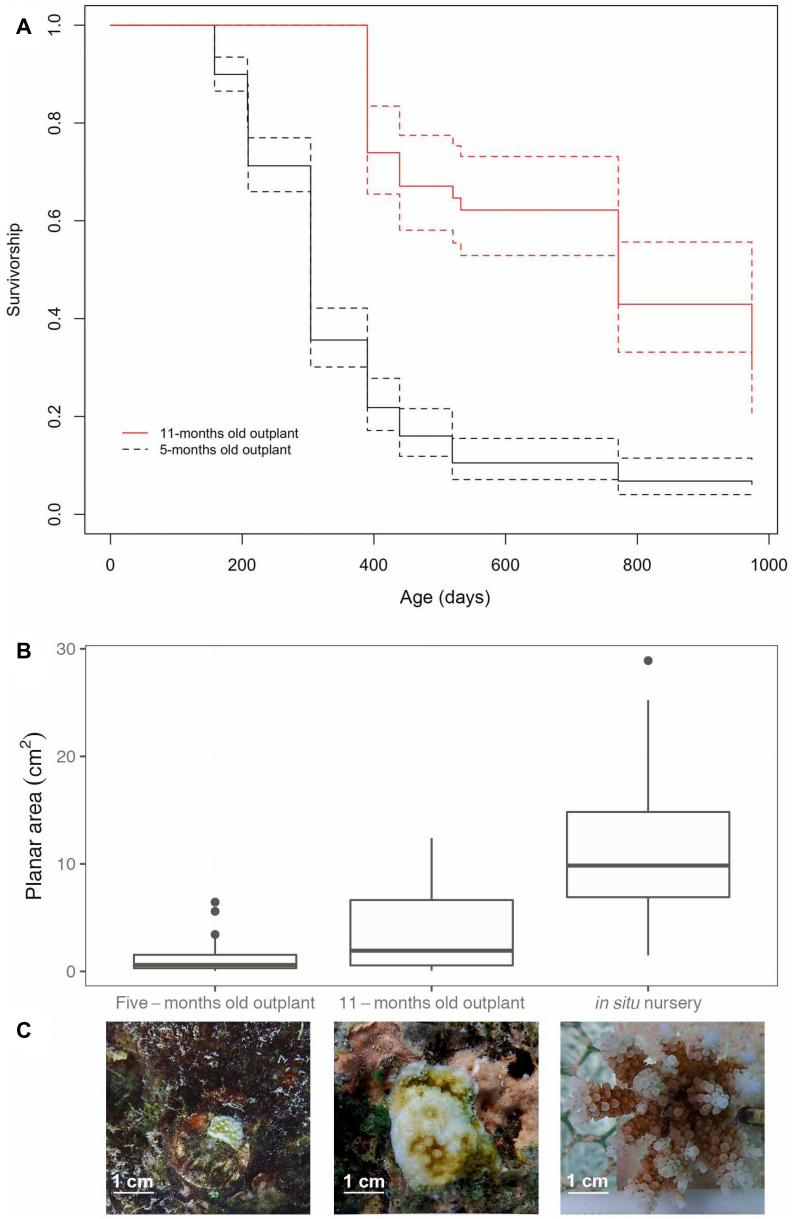

FIGURE 7 | (A) Survivorship curves for *Acropora digitifera* corals outplanted to the reef when colonies were 5-months (black line) and 11-months old (red line). Dashed lines indicate 95% confidence intervals. **(B)** Planar area comparison between colonies from the *ex situ* nursery that were outplanted to the reef at 5 and 11-months old or transferred to the *in situ* nursery at 13-months old. **(C)** Images showing representative corals in the different treatments at the same age (17-months old).

depression (Edmands, 2006; Baums, 2008). It also reduces logistical challenges associated with the collection and transport of colonies or gametes between populations (i.e., between geographically distant locations or countries). Conversely, a limitation of conducting SB within populations is the need to identify in advance sufficient numbers of distinct genets with the desired trait using appropriate stress trials, and to avoid breeding closely related colonies. Sufficient tagged colonies also need to survive, remain healthy, and be gravid at the predicted spawning time. An alternative to direct testing for a desired phenotype is the identification of biomarkers of stress tolerance (i.e., a specific lipid or protein constituents, immune profiles, genes, microbial or Symbiodiniaceae symbiont communities, etc.). However, as yet no biomarkers have been developed for any trait in corals and further research in this area is needed to develop these as tools. An option to overcome this hurdle is to create clonal broodstocks of colonies with desired traits either in an *in situ* nursery or at the parental reef. A combination of these two practices could increase the access to genets for SB, and even provide access to the same broodstock during consecutive years.

TABLE 2 | Cost of sexually propagating *Acropora digitifera* corals using a selective breeding framework with corals reared under *ex situ* nursery tanks until 11-months old when they were outplanted to the reef.

Category	Capital equipment	Consumables	Labor	Total	% total cost
Coral collection	$426	$785	$312	$1,521	7
Spawning to competency	$1,353	$803	$1,274	$3,430	15
Settlement	$181	$624	$213	$1,018	5
Ex situ nursery rearing	$1,592	$254	$575	$2,421	11
Outplanting at 11-months old	$347	$4,807	$2,010	$7,164	32
Monitoring and maintenance	$559	$3,525	$2,861	$6,945	31
Grand Total	$4,459	$10,798	$7,243	$22,500	100

The capital equipment is pro-rated over 5 years.

We limited our SB design to single pairwise crosses (only two parental colonies, **Figure 8.2A**), because the number of eggs available per colony limited the number of crosses that could be performed, while still producing sufficient colonies for outplant. To perform single pairwise crosses, broodstock needed to be collected and isolated in tanks before spawning, with all spawning and fertilization work carried out *ex situ* in relatively controlled laboratory conditions (**Figure 8.3B**). In some cases, this approach might not be possible, if for example, spawn collection has to be done *in situ* (**Figure 8.3A**), or if natal reefs are far from facilities where spawning and rearing will take place. The total number of pairwise crosses that can be achieved during a spawning event will depend on the extent of synchronous spawning among colonies and the workforce available for the collection, separation and washing of gametes during their viable time period (<2 h; Omori et al., 2001). In addition, in order to maximize the number of pairwise crosses produced requires that each cross is reared independently, and therefore the number of culture vessels can quickly escalate. This then requires increased workforce effort to manage the husbandry during the early post spawning period.

For studies where heritability of the trait has been reported with both sperm and eggs (i.e., Liew et al., 2020), then mass crosses (more than two selected parental colonies, i.e., colonies with relative high heat tolerance, **Figure 8.2B**) may be a better approach than carrying out many individual crosses. These can be done either by (a) pooling gametes of all colonies with the trait of interest to produce one culture that is divided into replicate larval rearing tanks, or by (b) pooling the sperm of several colonies to fertilize the eggs of a single colony. An advantage of pooling gametes is that corals can be allowed to spawn together *en masse* in a single tank reducing the work involved in collecting and separating bundles from many individual colonies. However, culture viability can be compromised by a modest percentage of unfertilized eggs that could originate from a single colony (Pollock et al., 2017), or the resulting larvae could be closely related if one of the genets dominates the pool and drives fertilization. Alternatively, pooling the sperm of several colonies to fertilize eggs from one colony requires collection, separation, and washing of gametes as previously described for pairwise crosses. The benefits of this procedure are that it can be replicated with eggs from all donor colonies, resulting in several distinctive crosses with potentially higher fertilization success, as the concentration of gametes can be better controlled. However, whilst logistical constraints during spawning and fertilization periods are eased through either colony or sperm pooling, a significant drawback to these approaches is that resulting cohorts produced are a mix of either half or non-related kin. Long-term post-settlement survival in these kin groups may be lower, compared to fully related offspring, due to negative allogenic interactions following developmental onset of the corals' immune response (Puill-Stephan et al., 2012). Therefore, trade-offs occur between increased work and facilities required to produce large numbers of pairwise crosses versus simplified logistics and reduced larval rearing costs but potentially increased post settlement mortality.

Scientists have successfully conducted CLP for three decades, and great progress has been achieved in controlling steps associated with the early stages, i.e., controlling spawning times (Craggs et al., 2017), fertilizing gametes *en masse*, and rearing larvae to obtain coral colonies (Guest et al., 2010; Randall et al., 2020). Thus, one of the more challenging steps in CLP is to settle larvae efficiently onto SUs and enhance their survivorship and growth until outplant (**Figure 8.4**). Our results show an average of four corals per SU (1.3 settlers per cm^2) at 10 days after settlement, resulted in at least one colony after four months of *ex situ* husbandry rearing. Despite coral post-settlement survivorship being influenced by settlement density and husbandry conditions (Conlan et al., 2017; Cameron and Harrison, 2020), our results provide a useful guideline for optimal settlement densities when using SUs for CLP. For SUs outplanted at different ages, similar analyses are needed to provide information on the minimum number of larvae to settle to obtain one colony per SU at the age of outplant. Furthermore, restoration initiatives will benefit from knowing the optimal number of larvae per SU that will maximize the probability of obtaining a colony that attains sexual maturity, a factor that will be dependent on the overall survivorship of the outplant. An optimal use of available competent larvae for settlement will be a defining factor of the efficiency of the effort. However, the production of larvae is one of the steps which incurs the lowest costs in the framework (**Table 2** and **Supplementary Material 14**) so efficiency and cost-efficiency may not go hand in hand. Settling more larvae per SU than needed will waste larvae, whereas, settling too few larvae per SU lowers yield and increases costs. Evidence indicates that low larval settlement densities

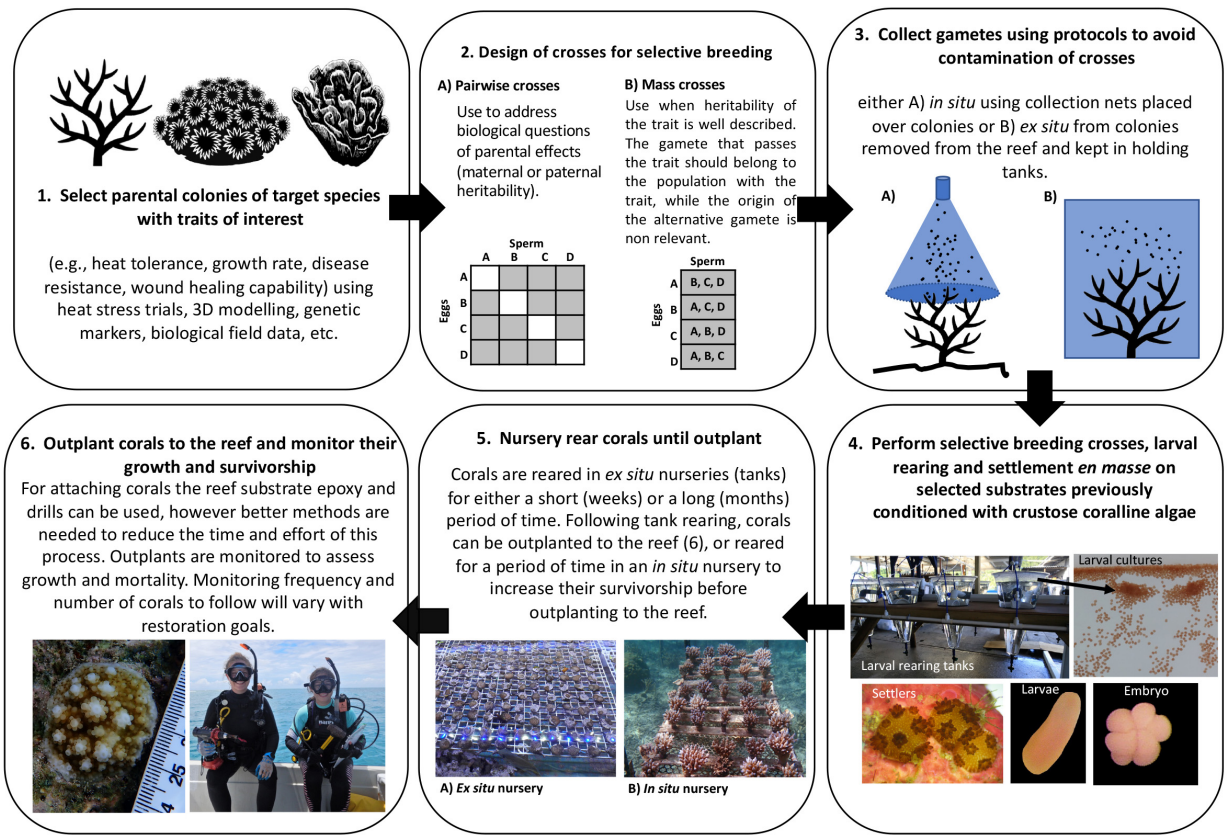

FIGURE 8 | Practical framework for coral reef restoration ecology using selective breeding.

can compromise the production of SUs with at least one colony at the time of outplanting due to high post-settlement mortality, while high settlement densities can compromise survivorship due to density dependent effects (Doropoulos et al., 2017; Cameron and Harrison, 2020). Additionally, high larval densities promote the formation of chimeras, a factor that needs further research as their implications on colony growth and survivorship are still understudied (however see Rinkevich, 2019; Huffmyer et al., 2021).

Husbandry of corals during the early post-settlement stages is a key step for successfully conducting CLP as it has a significant impact on growth and survivorship. Techniques for rearing settlers until they can be outplanted to the reef in sufficiently large numbers remain largely experimental. Early survivorship after settlement is the primary bottleneck in CLP for improving cost-effectiveness. Our results show that extending *ex situ* nursery times increased both survivorship and growth rates of outplants and reduced the costs of the production of colonies. Husbandry during the early months has been proven to increase coral survivorship (Baria et al., 2010; Guest et al., 2014; Craggs et al., 2019) and growth (this study) by reducing predation and competition pressures (Doropoulos et al., 2016; Gallagher and Doropoulos, 2017). Moreover, *ex situ* rearing can enhance colony growth by controlling light, water and food quality, and by co-culturing corals with herbivores to limit algal overgrowth (Craggs et al., 2019) and reduce costs (**Figure 8.5A**, **Table 2**, and **Supplementary Material 14, 15**). However, there may also be drawbacks associated with longer nursery times such as: (1) acquisition of a Symbiodiniaceae community that differs from the one at the outplant site (Baums et al., 2019), (2) potential fitness consequences of plastic and epigenetic changes due to exposure to different environmental conditions during nursery rearing (Parkinson and Baums, 2014), and (3) development of pathogens or diseases during husbandry (Sheridan et al., 2013).

Many of the disadvantages encountered in *ex situ* nurseries (see above and van Woesik et al., 2021) can be avoided under *in situ* conditions (**Figure 8.5B**), especially if the location of the nursery is close to the outplant sites. However, the costs associated with *in situ* (**Supplementary Material 18**) husbandry are considerably higher than *ex situ* conditions (**Table 2**), given the logistic and practical constraints associated with working underwater. The location of the *in situ* nursery in a place with similar environmental conditions to the outplant site, in a shallow area with good water quality that is protected from storms, and that is easy to access can enhance the survivorship and growth of the corals and reduce costs. Local knowledge and historical environmental data will improve the chances of locating appropriate *in situ* nurseries sites. However, one major limitation of *in situ* nurseries is that they are prone to damage due to environmental stressors (i.e., temperature fluctuations and

storms) and human activities (e.g., diver or anchor impacts). Moreover, enhanced growth rates at *in situ* nurseries can be associated with reductions in skeletal densities (Pratchett et al., 2015), which may disadvantage colony performance once outplanted to the reef.

The outplanting phase was the most expensive activity of this framework, highlighting the need for technical development (**Figure 8.6**) if restoration is to be scaled up. The method used for outplanting corals in this study is not suitable for larger scale restoration and should not be considered as such as it is laborious, slow, and costly and only appropriate at a scale of tens to hundreds of square meters (Guest et al., 2014). The outplanting methodology should minimize the use of tools and attachment materials (i.e., Coralclip®; Suggett et al., 2019). SUs should be designed so that they are easy to transport, can be rapidly and easy deployed, can be readily attached to the reef substrate (i.e., tetrapods; Chamberland et al., 2017) and, if possible be made of sustainable and ecologically friendly constituents (i.e., sustainable cement; de Brito and Kurda, 2021). The design of SU should enhance coral survivorship until they reach an escape size (i.e., with micro-refugia to protect colonies from grazing pressure) and improve the success of early outplant, reducing husbandry time. Interdisciplinary teams of ecologist, aquarists and engineers are key to developing novel designs of SUs that can be easily deployed in different reef environments (i.e., reef crest and reef flat), which is a critical factor for CLP and SB techniques to be adopted as a management strategy of coral reefs.

The cost analysis reveals that extending husbandry time has a major impact on the efficiency of the propagation effort and reducing the cost of 2.5-year-old colonies five times as a result of early survivorship increases. Total costs could be reduced considerably if post-outplant survivorship is increased at younger ages, thus decreasing husbandry times, hence innovation in this area is fundamental to reduce total costs. Likewise, several strategies could be adopted to reduce costs associated with monitoring, for example, (a) surveying only a representative subset of outplants, (b) developing a citizen science program which incorporates the local and tourist community during the monitoring phase (Sinclair et al., 2021), and (c) use of innovative technologies like photogrammetry to monitor large reef areas (∼250 m) while minimizing time underwater (Lechene et al., 2019). Cost breakdown of CLP is not straightforward, owing to several factors that vary between coral species, sites, facilities and countries. Yet, doing the exercise of estimating such expenses accounting for types of costs (i.e., capital equipment, consumables, and labor) provides a quantitative method to identify the steps that need further development to improve overall efficiency and reduce costs. Reef rehabilitation initiatives using CLP under a SB approach will effectively become a management strategy to promote adaptation and resilience of reefs in the Anthropocene when techniques are proven to be cost-effective.

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

AH, JCB, AJE, PP, ES, AT, and JRG contributed to conception and design of the study. AH and AJE organized the database. AH, AJE, LL, and ES performed the statistical analysis. AH wrote the first draft of the manuscript. AJE, LL, ES, and JRG wrote sections of the manuscript. All authors contributed to data collection, manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.669995/full#supplementary-material

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Genotype, Nursery Design, and Depth Influence the Growth of *Acropora cervicornis* Fragments

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Growing fragments of corals in nurseries and outplanting them to supplement declining natural populations have gained significant traction worldwide. In the Caribbean, for example, this approach provides colonies of *Acropora cervicornis* with minimal impacts to existing wild colonies. Given the impetus to scale up production to augment limited natural recovery, managers and researchers should consider how the design and location of the nurseries affect the growth of different genotypes of corals and the effort required for maintenance. To elucidate such influences, we grew fragments of different genotypes (five varieties) on differing structures (trees and frames) at two depths (6–8 and 16–18 m). The sum of the lengths of all branches or total linear extensions (TLEs) and accumulation of biofouling were measured over 198 days from May to December 2016 to assess the growth of fragments and the effort required to maintain nurseries. TLEs for all fragments increased linearly throughout the incubation period. Mean daily incremental growth rates varied among the genotypes, with one genotype growing significantly faster than all others, two genotypes growing at intermediate rates, and two genotypes growing more slowly. Mean daily incremental growth rates were higher for all genotypes suspended from vertical frames at both sites, and mean daily incremental growth rates were higher for all fragments held on both types of nurseries in deeper water. If linear growth continued, a fragment of the fastest growing genotype held on a frame in deeper water was estimated to increase the sum of the length of all its branches by an average of 88 cm y^{-1}, which was over two times higher than the estimated mean annual growth rate for a fragment of the slowest growing genotype held on a tree in shallow water. Nurseries in deeper water had significantly less biofouling and appeared to be buffered against daily fluctuations in temperature. Overall, the results demonstrated that increased production and reduced maintenance can result from considering the genotype of fragments to be cultured and the design and location of nurseries.

Keywords: coral restoration, staghorn coral, nursery trees, nursery frames, temperature, biofouling, coral propagation

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INTRODUCTION

Historically, the staghorn coral *Acropora cervicornis* was a dominant foundation species that provided much of the rugosity and structural complexity on Caribbean coral reefs (Gilmore and Hall, 1976; Tunnicliffe, 1981; Aronson and Precht, 1997; Alvarez-Filip et al., 2009). This species dominated forereef and shallow spur and groove areas throughout much of the Caribbean because of its rapid growth rates and capacity for asexual reproduction (Tunnicliffe, 1981; Aronson and Precht, 1997). However, the abundance of staghorn corals has declined substantially throughout the Caribbean, with some areas losing more than 97% of historical cover over the past five decades (Acropora Biological Review Team, 2005). This precipitous decline in Caribbean acroporids resulted from white band disease (WBD), which was first observed in 1976 (Gladfelter, 1982), followed by cascading effects on food webs arising from the demise of the long-spined sea urchin *Diadema antillarum* in the early 1980s and damage caused by hurricanes (Hughes and Connell, 1999; Williams and Miller, 2005). Although modest recovery of staghorn corals has been reported in a few areas of the Caribbean, a rapid natural recovery has been inhibited by the persistence of WBD, more frequent and severe bleaching events, and competition with macroalgae due to insufficient grazing by the reduced numbers of *D. antillarum* (Aronson and Precht, 2001; Precht et al., 2002).

In an attempt to enhance remaining wild populations and regenerate degraded reefs, acroporid nurseries have proliferated throughout the Caribbean in the last two decades (Edwards and Gomez, 2007; Johnson et al., 2011; National Marine Fisheries Service, 2015). Initial nurseries in the early 2000s employed fragments of coral attached to concrete blocks or polypropylene lines (Johnson et al., 2011; Young and Schopmeyer, 2012). New techniques reduced mortality and improved growth rates by placing nurseries in sheltered environments and raising corals above the substrate (Bowden-Kerby, 2001; Bowden-Kerby et al., 2005; Griffin et al., 2012). Nurseries that held fragments in the water column minimized direct predation by corallivorous snails, *Coralliophila abbreviata*, and fireworms, *Hermodice carunculata* and increased access to food (Young and Schopmeyer, 2012). Reducing the damage to coral tissue caused by predation also mitigated transmission of WBD and other waterborne pathogens (Sussman et al., 2003; Gignoux-Wolfsohn et al., 2012). Today, identifying and propagating genetically distinct lineages of corals has become standard practice for nurseries (Johnson et al., 2011). Furthermore, nurseries now produce multiple fragments of coral for outplanting from small portions of wild colonies, and they act as a repository of local genets in the event donor colonies are lost (Schopmeyer et al., 2012). Overall, enhancing genetic diversity in outplants represents a means of improving spatial connectivity that is a critical component of regional restoration (Lirman and Schopmeyer, 2016). At the regional scale, staghorn coral populations have become severely isolated, so propagation of multiple genets improves the likelihood of restoring the successful *in situ* sexual reproduction that fostered genetic diversity in historical populations (Drury et al., 2017).

Nurseries for corals serve as crucial intermediary steps between collection of fragments from wild colonies and outplanting (Johnson et al., 2011). Despite the increased use of nurseries in the Caribbean, there is currently a dearth of information regarding the influences of genotypes, designs of nurseries, and depth of deployment on the growth of the fragments and the need for maintenance (Young and Schopmeyer, 2012; Schopmeyer et al., 2017). For example, PVC trees have been one of the most popular structures for suspending coral fragments in the water column (Nedimyer et al., 2011). However, restoration after the grounding of the T/V Magara in Puerto Rico suggested that planar, vertical frames placed in deeper water (15–18 m) offered significant advantages (Griffin et al., 2012). Specifically, the fragments on frames in deeper water grew significantly faster and the frames accumulated biofouling at a slower rate than had been reported elsewhere (Quinn and Kojis, 2006; Lohr et al., 2015; Schopmeyer et al., 2017). In addition, experimental work off Little Cayman Island indicated that splitting coral fragments could increase the numbers available for outplanting dramatically; however, limited replication in that study precluded evaluation of variation in growth rates among genotypes (Lohr et al., 2015).

Although information can be compared across studies, to our knowledge, a simultaneous comparison of growth rates for different genotypes attached to nurseries with different designs that were deployed at different depths has not been reported. This study used a field experiment conducted off Little Cayman Island to address this gap in knowledge and inform best practices for culturing fragments of *A. cervicornis*.

MATERIALS AND METHODS

Experimental Design: Sites, Nurseries, and Genotypes

Nurseries were deployed over the course of 2 days at the end of May, and measurements were collected for 198 days from June 2016 to December 2016 at two sites along the northern coast of Little Cayman Island (**Figures 1A,B**). The shallow site was located approximately 150 m from the shoreline in 6–8 m of water on a hardpan plain between the fringing backreef and the beginning of the spur and groove formation (**Figure 1C**). This site was characterized by sparse, small coral heads and gorgonians, and it lacked high densities of scleractinian corals or large sponges. Mixed schools of ocean surgeonfish, *Acanthurus tractus*, and doctorfish, *Acanthurus chirurgus*, frequently grazed on the nurseries. The deep site was established approximately 100 m north of the shallow site in 16–18 m of water (**Figure 1C**). This site was located in 50 m of sand and rubble at the deeper terminus of a shallow groove in a spur and groove formation. This sandy area was bordered by reef spurs to the east and west and a sandy plain to the north. The adjacent spurs were dominated by corals in the genera *Orbicella*, *Pseudodiploria*, and *Porites*, with *Diploria* and *Agaricia* also found in relatively high abundance. The area of sand and rubble was mostly barren, with occasional small fish in the genus *Labridae* found near the substrate. Several fish species were seen along the reef spurs, and

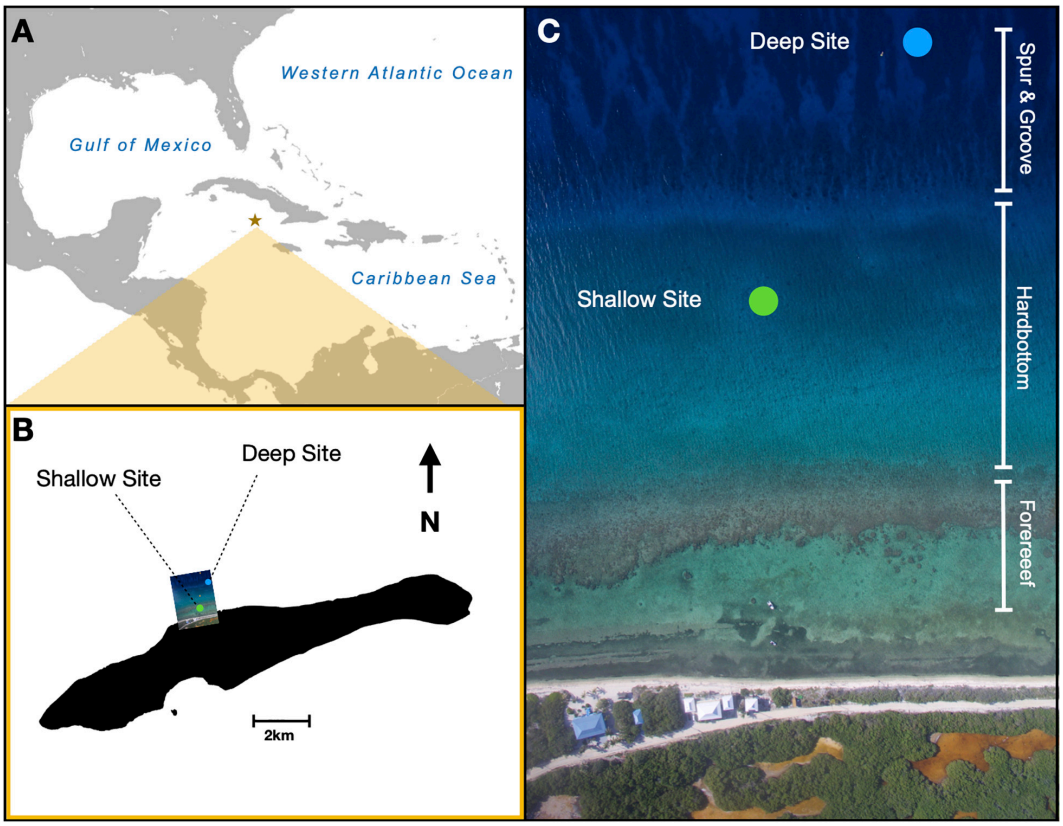

FIGURE 1 | Locations of nurseries for staghorn coral *Acropora cervicornis* examined in this study. Nursery sites were in the **(A)** central Caribbean on **(B)** the north side of Little Cayman Island. **(C)** Arial imagery shows the specific locations of the shallow (6–8 m) nursery site located in hardbottom habitat and deep (15–18 m) nursery site located on sand in between spur and groove reef.

large schools of creole wrasse, *Clepticus parrae*, were observed in the nearby water column.

Two types of nurseries were used: trees and frames. Trees were 2 m in height by 1 m wide, with a single vertical column kept upright by a buoy and nine horizontal PVC branches from which the corals were suspended (**Figure 2A**). Similar nurseries have been deployed throughout the Caribbean (Johnson et al., 2011; Nedimyer et al., 2011). Frames were PVC rectangles anchored at two points and buoyed at the two opposing points so that the frame remained perpendicular to the substrate (**Figure 2B**). Each frame was 3.0 m wide by 1.5 m tall, with a center brace and five lines of 400-lb test monofilament strung horizontally. Originally, this design was employed as nurseries for staghorn corals in Puerto Rico (Griffin et al., 2012).

Three replicate nurseries of each design were placed at both sites (total number of nurseries = 12). Stainless steel pins were used to attach trees and frames to the hardbottom substrate at the shallow site (**Figure 2A**). Concrete blocks partially buried in sand anchored trees and frames at the deep site (**Figure 2B**). Nurseries at the shallow site were placed at least 20 m away from any pre-existing structures, and each nursery at both sites was at least 5 m away from its neighbors.

Each nursery was populated with 36 fragments of staghorn coral from five genotypes previously identified as genetically distinct (Drury et al., 2017) over 2 days in late May 2016. Genotypes were identified by colored beads, with nine yellow, nine red, nine green, five black, and four blue fragments attached to each structure. Fewer black and blue genotypes were used due to the limited number of fragments available. On trees, four haphazardly selected coral fragments were hung from each of the nine branches (n = 36 fragments). On frames, the 36 coral fragments were haphazardly distributed among the 50 possible attachment sites (i.e., 10 potential sites across each of the five horizontal lines). All 432 fragments of coral were attached using 100-lb test monofilament line and aluminum crimps (**Figure 2C**). Fragments were selected to be similar in size as determined by summing the lengths of all their branches, i.e., measuring total linear extensions (TLEs). The mean and standard error of initial measurements was 12.1 $\pm$ 4.1 cm.

Each nursery also had three, 4-cm-long pieces of PVC pipe attached in the same way as fragments of coral (**Figure 2D**). Each piece of PVC was weighed to the nearest 0.01 g before deployment and identified by a uniquely numbered metal tag so that changes in weight due to biofouling could be determined.

Data Collection and Maintenance

Growth of coral fragments was quantified by measuring the lengths of all branches comprising each fragment to the nearest

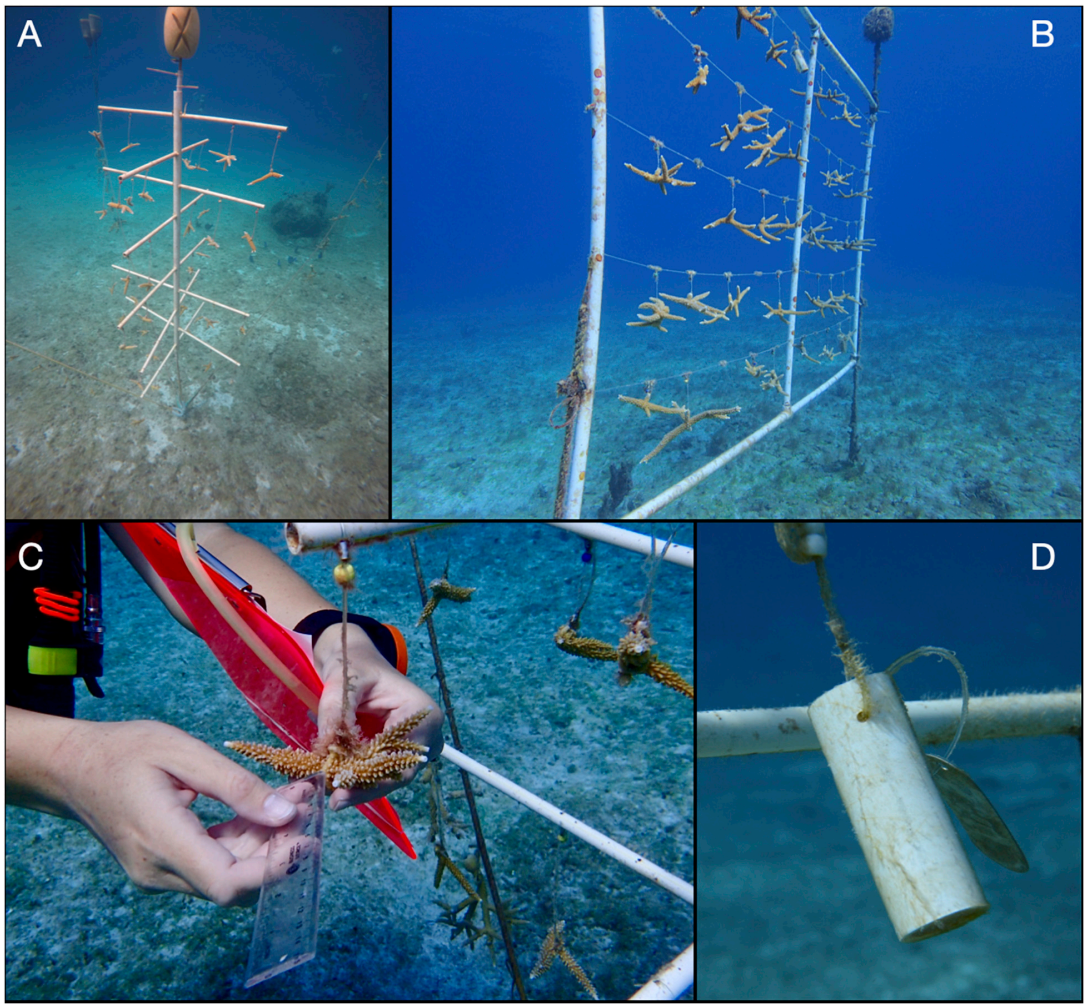

FIGURE 2 | Digital images of nurseries and data collection in this study. Two types of nurseries were used: **(A)** a tree that comprised a vertical column and nine horizontal PVC branches or a **(B)** frame that comprised PVC in a vertical rectangle with monofilament strung horizontally. **(C)** Total linear extensions of coral fragments measured by divers. Accumulation of biofouling was quantified on **(D)** 4-cm-long piece of PVC attached to the nurseries.

0.5 cm and summing those measurements to yield TLEs (**Figure 2C**). Measurements were taken *in situ* by SCUBA divers monthly from June 1 through August 2016, and subsequently, every other month through December 15, 2016, for a total of five sets of measurements. Data sheets included TLEs from the previous dive to minimize measurement error and prompt a search for broken branches should there be a reduction or no change in a TLE.

Temperature was sampled contemporaneously at the shallow and deep sites with Onset HOBO® Pendant Temperature/Light 64K data loggers. Readings were recorded every 30 min for the duration of the study. Data loggers were downloaded *in situ* every 2–3 months using an Onset HOBO Waterproof Shuttle.

In August 2016, the pieces of PVC that had been deployed in June were removed and placed into individual Ziploc® bags to minimize the loss of accumulated biofouling during handling and transportation. In the laboratory, each section was weighed to the nearest 0.01 g after 8 h of drying in ambient conditions.

Maintenance was performed twice monthly if weather permitted. Divers used cloth gloves to remove fouling organisms from all nursery structures, with the exception of the pieces of PVC deployed to accumulate biofouling.

Analysis of Data

The TLEs were analyzed in two ways. First, TLEs for replicate fragments of coral in each of the 20 different combinations of genotype, depth, and type of nursery were regressed against days since installation to determine if growth remained linear. Second, daily incremental growth rates were derived from measurements of TLEs by taking the difference between two successive measurements and dividing it by the number of days between them. Data for fragments that showed loss of skeletal material were omitted, which accounted for less than 5% of all coral fragments. The resulting growth rates were analyzed with a permutation analysis of variance (PERMANOVA) computed in PRIMER 6 (Anderson et al., 2008). The PERMANOVA treated

genotype (yellow, red, green, black, or blue), depth (shallow or deep), and type of nursery (tree or frame) as fixed factors. Three nested (i.e., random) factors accounted for the presence of repeated measures of individual fragments of coral: (1) the three replicate nurseries installed at each depth, (2) each individual fragment of coral hung from every nursery, and (3) the number of days since installation for each observation. The hierarchical portion of the analysis had replicate nested in the interaction of the three fixed factors, individual coral fragments nested in replicates, and days of observation nested in individuals. Where appropriate, additional pairwise, permutation analyses were performed to identify significant differences among levels of fixed factors or their interactions.

High-resolution temperature data (30-min intervals) were used to identify consistent differences between the shallow and deep sites that may have influenced growth rates. A faulty recorder resulted in temperature data being available only after July 30. The data were used to calculate mean daily temperature over the intervals between measurements of TLEs, and the resulting data were analyzed using a paired *t*-test. The high-resolution data were also examined to identify the frequency and duration of potential thermal stress based on a 31°C threshold drawn from the literature (Shinn, 1966; Acropora Biological Review Team, 2005; Manzello et al., 2007).

Amounts of accumulated biofouling were used to characterize the need for maintenance of nurseries. Amounts of accumulated biofouling at the shallow and deep sites were calculated as the difference between the weights of the pieces of PVC after and before being deployed on nurseries. These differences were analyzed with a Welch's *t*-test that accounted for unequal variances.

RESULTS

All 20 regressions of TLEs against days since installation were significant at $p < 0.001$, and they indicated that growth rates remained relatively constant (**Supplementary Figure 1**). In general, TLEs for individual fragments became more variable as the experiment progressed. Out of the 10 steepest slopes, which corresponded to the highest growth rates, 80% involved the yellow, black, or blue genotypes; 60% involved fragments on frames; and 80% involved fragments held in deeper water.

These trends were confirmed by the PERMANOVA, which showed that mean daily incremental growth rates varied significantly among replicate nurseries (**Table 1**). Among the 60 replicate nurseries, mean daily incremental growth rates ranged from 0.07 to 0.28 cm day^{-1} (**Supplementary Figure 2**). Out of the 20 highest growth rates, 90% involved the yellow, black, or green genotypes; 55% involved fragments held on frames; and 85% involved fragments held in deeper water. These results highlighted fine-scale variation, which should be considered when culturing fragments of coral. Although variation among replicate nurseries provided some useful and useable insights, the other F-ratios accounted for this variation and provided highly relevant insights. Therefore, the significant effects of genotype, depth, and type of nursery were investigated (**Table 1**; please see pp. 46–48 in Anderson et al., 2008).

Regardless of the type of nursery or site where they were located, genotypes grew at significantly different rates (**Figure 3A**). *Post hoc* tests indicated that the yellow genotype had the highest daily incremental growth rate (mean ± standard error = 0.21 ± 0.006 cm day^{-1}), red and blue genotypes grew slowest (0.13 ± 0.005 and 0.11 ± 0.005 cm day^{-1}, respectively), and black and green genotypes displayed intermediate daily incremental growth rates (0.17 ± 0.005 and 0.16 ± 0.005 cm day^{-1}, respectively). Depth and type of nursery also had significant effects on growth rates. Fragments at the deep site (0.18 ± 0.004 cm day^{-1}) grew significantly faster than fragments held at the shallow site (0.13 ± 0.003 cm day^{-1}; **Figure 3B**). Although the difference was less pronounced, growth rates were also significantly higher for fragments on frames (0.17 cm day^{-1} ± 0.004 SE) compared to fragments on trees (0.15 cm day^{-1} ± 0.003; **Figure 3C**).

Growth rates at the two sites may have been affected by water temperatures, which did differ. Mean daily water temperatures at the shallow site were significantly higher than those at the deep site ($t_{138} = 10.12$, $p < 0.001$). However, the mean values differed by ∼0.1°C (mean ± standard error of 29.7 ± 0.08°C for the shallow site and 29.6 ± 0.08°C for the deep site), so average

TABLE 1 | Results of permutation analysis of variance for incremental growth rates.

Terms for F-ratios	Source of variation	Degrees of freedom	Sums of squares	Mean squares	Pseudo-*F* ratios	*p*	Unique Permutations	Terms in *F*-ratios
1	Ge	4	2,473.5	618.4	18.76	0.001	998	1/(8 + 9)
2	De	1	1,741.9	1,741.9	55.16	0.001	998	2/(8 + 9)
3	Nu	1	141.3	141.3	4.48	0.043	998	3/(8 + 9)
4	Ge × De	4	72.7	18.2	0.55	0.702	999	4/(8 + 9)
5	Ge × Nu	4	306.2	76.6	2.32	0.069	999	5/(8 + 9)
6	De × Nu	1	5.3	5.3	0.17	0.683	998	6/(8 + 9)
7	Ge × De × Nu	4	102.7	25.7	0.78	0.544	998	7/(8 + 9)
8	Rep(Ge × De × Nu)	40	1,337.2	33.4	2.20	0.001	997	8/9
9	Ind[Rep(Ge × De × Nu)]	353	5,370.6	15.2	1.12	0.094	993	9/10
10	Day{Ind[Rep(Ge × De × Nu)]}	1,239	16,880.0	13.6	No test			
	Total	1,651	28,643.0					

Ge, genotype; De, depth; Nu, nursery type; Rep, replicate nursery; Ind, individual coral fragment; Day, day of observation.

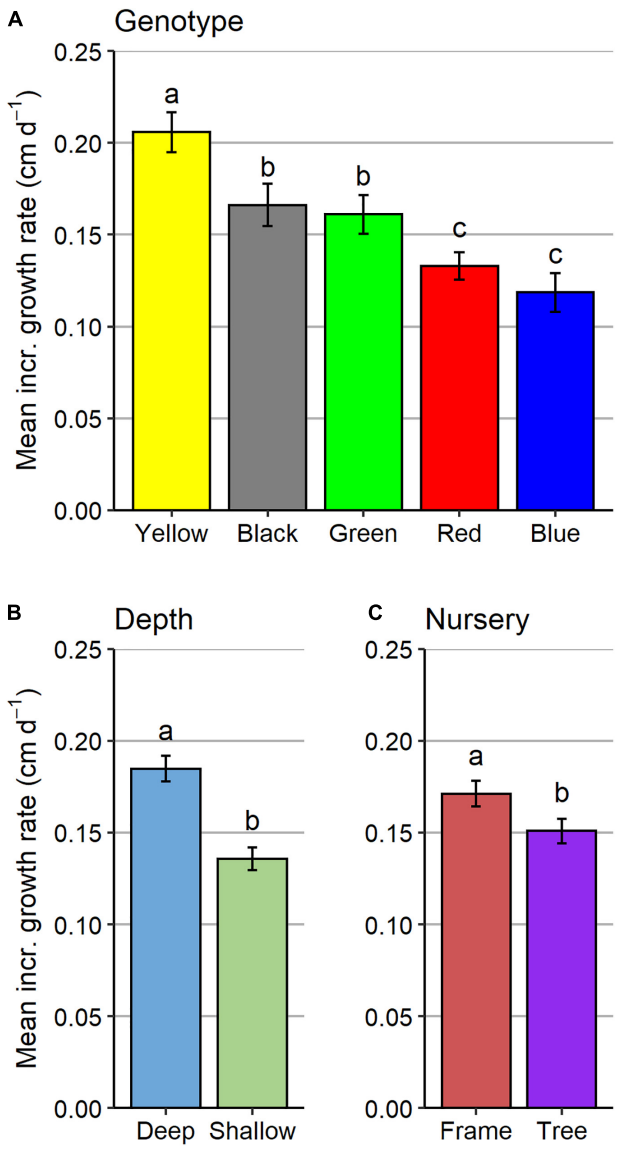

FIGURE 3 | Mean incremental growth rates ± standard errors (SE) for **(A)** different genotypes, **(B)** depths, and **(C)** nurseries. Incr., incremental.

temperatures may not have been the most relevant factor. Further evidence that the shallow site experienced warmer temperatures was derived from the data collected every 30 min, particularly during the warmer months of July–September (**Figure 4**). During these months, temperatures surpassed 31°C at the shallow site on 28 days, which included two runs of 12 and 13 consecutive days, respectively (**Figure 4A**). In contrast, temperatures at the deep site surpassed 31°C only once (**Figure 4B**) over the same time period. In addition, the peak daily temperature was greater than 1°C warmer at the shallow site on 5 days in August (**Figure 4C**). Despite relatively high temperatures, bleaching was not observed for any of the fragments at either site.

Algae accumulated on 12 pieces of PVC at the shallow site and 12 pieces of PVC at the deep site for 3 months. Upon collection, algal biomass on pieces from the shallow site were significantly higher (t_8 = 4.86, p = 0.001), with mean dry weights ± standard errors being 1.03 ± 0.15 g for the shallow site and 0.28 ± 0.02 g for the deep site.

DISCUSSION

To our knowledge, this study is the first to simultaneously examine the influences of genotype, design of nurseries, and depth on the growth rates of fragments of staghorn coral, along with efforts to evaluate potential temperature stress, rates of biofouling, and time required for maintenance. We recorded significantly higher mean daily incremental growth rates for fragments of the yellow genotype (24% higher than the next fastest growing genotype and 74% higher than the slowest growing genotype), fragments held at the deep site (36% higher than fragments held at the shallow site), and fragments held on frames (13% higher than fragments on trees). Temperature data suggested that nurseries at the shallow site were exposed to potentially stressful sea surface temperatures during warmer months, which correlated with a 3.5 × higher rate of accumulation of algal biomass at the shallow site. In our experience, less biofouling at the deeper site led to less time expended on maintenance. Collectively, these results point to the value of considering multiple factors when culturing coral fragments to support restoration of coral reefs.

Out of all the influences examined, genotype yielded the greatest variation in growth rates of fragments of coral, with the fastest growing genotype extending at a rate that was nearly twice that of the slowest growing genotype. In addition, these differences in growth rates remained consistent regardless of which depth or type of nursery was employed. Differences in growth rates among genotypes were expected because they had been reported in other studies using nursery-reared corals held in the water column (Lohr et al., 2015; O'Donnell et al., 2017).

Since the goal of coral nursery propagation is to provide genetically diverse and robust coral populations that ultimately survive and thrive after outplanting (Baums et al., 2010), growth rates should not be the only consideration. Slower growing corals may have desirable characteristics, such as thermal tolerance (Jones and Berkelmans, 2010) or disease resistance (Hunt and Sharp, 2014). Such characteristics and survival rates have not been evaluated for staghorn coral off Little Cayman Island; therefore, we support the recommendation made by Shearer et al. (2009), regarding the value of culturing numerous genotypes to establish and maintain sufficient genetic diversity. In summary, further work off Little Cayman Island should track survival and growth after outplanting for more than five genotypes.

Three key and potentially interrelated findings from this study were the higher mean daily incremental growth rates recorded for fragments of coral held at the deep site, the reduced amount of biofouling at the deep site, and the potential for less thermal stress at the deep site. For example, linear extrapolation of growth rates at the deeper site yielded mean growth rates of 66 cm y^{-1}. Enhanced growth may have resulted from less biofouling, with approximately 70% less biofouling accumulating at the deep site (**Figure 5**). The enhanced growth and reduced biofouling

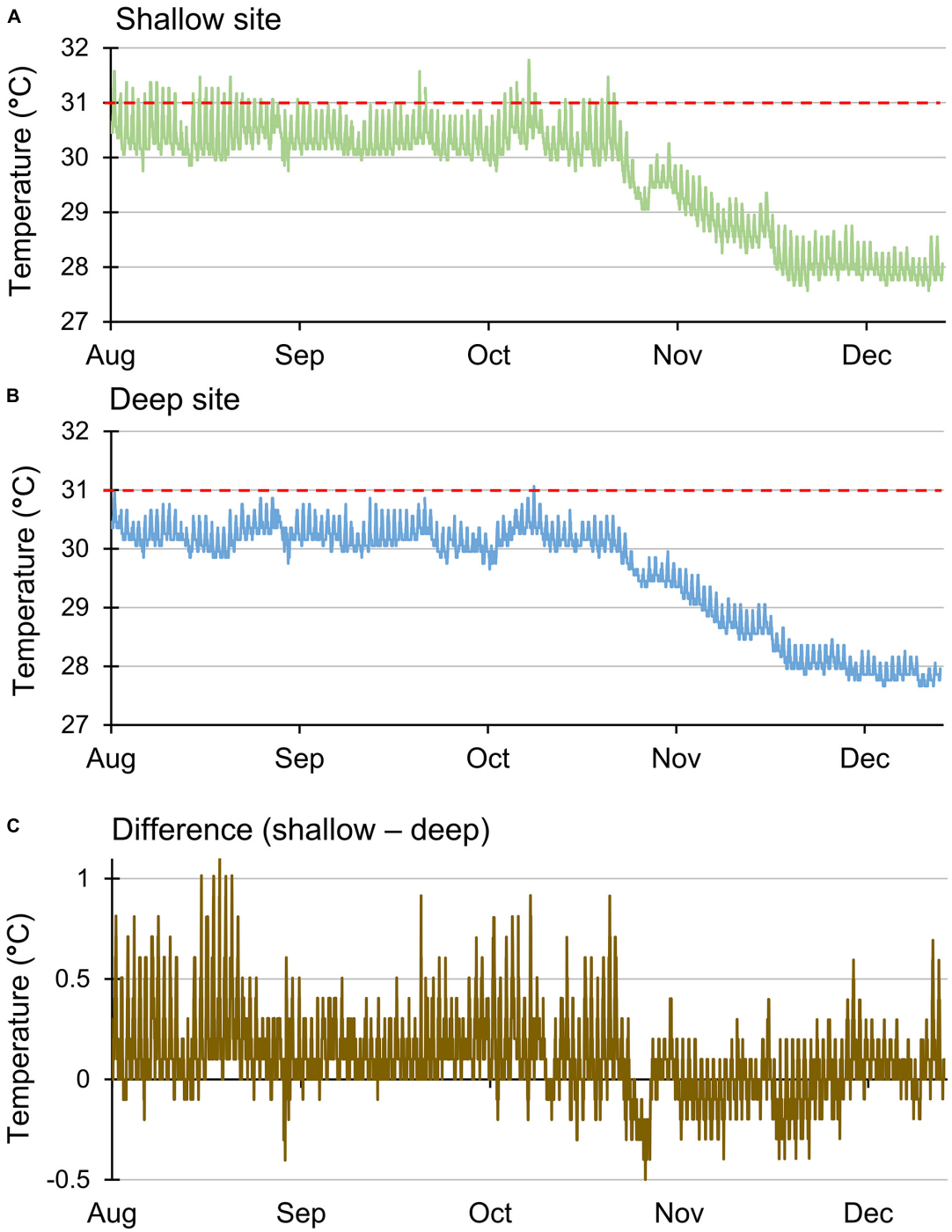

FIGURE 4 | Sea water temperature recorded every 30 min located at a **(A)** shallow site (6–8 m), **(B)** deep site (15–18 m), and **(C)** differences between those temperatures. Red line indicates the 31°C threshold for stress.

also may have resulted from consistently cooler temperatures at the deep site. In terms of enhanced growth, fragments of coral may have experienced reduced thermal stress as evidenced by fewer maximum daily temperatures above 31°C, although bleaching, evidence of severe thermal stress, was never observed. The cooler temperatures may also have combined with lower levels of light at the deep site to inhibit the growth of algae, which may have promoted more rapid growth of coral fragments and reduced the time required for maintenance of nurseries. These results aligned with similar observations of lower maintenance and higher growth rates reported for fragments of coral held on frames in deep water during preparations for restoring damage from the grounding of the T/V Magara (Griffin et al., 2012). However, the results contradicted previous reports of higher

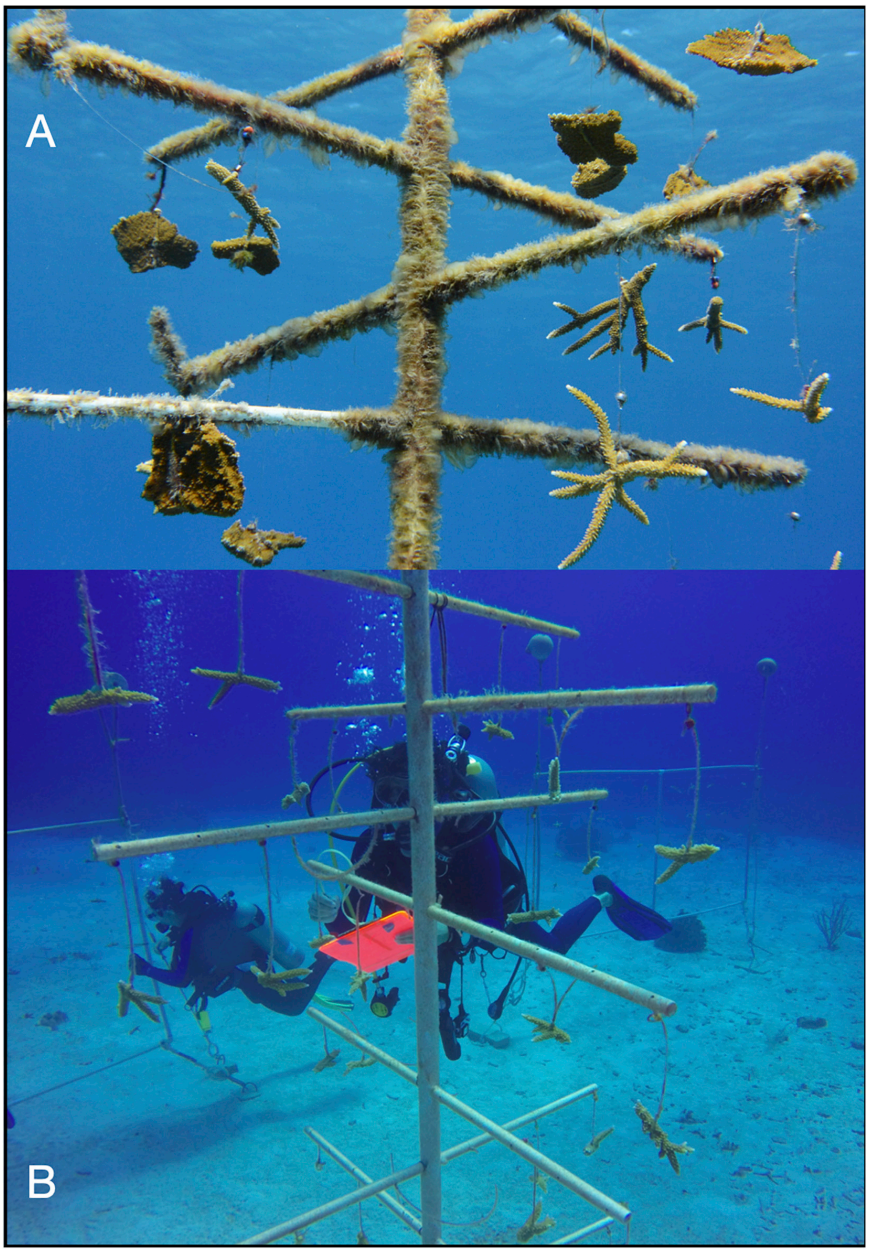

FIGURE 5 | Accumulation of biofouling on **(A)** a nursery at the shallow site and **(B)** a nursery at the deep site after approximately 1 month.

linear extension rates at shallower depths (Wellington and Glynn, 1983; Gladfelter, 1984; Huston, 1985), which were attributed to increased exposure to light. Furthermore, thresholds for optimal growth and onset of stress are likely to vary among locations and genotypes (Glynn, 1990; Knowlton et al., 1992; Edmunds, 1994; Rowan et al., 1997; Berkelmans, 2002; Manzello et al., 2007). In summary, our findings regarding the potential benefits of siting nurseries in deeper water require further evaluation that includes cultured fragments of coral at multiple sites and multiple depths.

The design of nurseries also influenced growth rates, the efficiency of data collection, and maintenance. In this study, even the slowest growing genotypes exhibited growth rates that were markedly higher than many corals grown in nurseries that were closer to the bottom (O'Donnell et al., 2017; Schopmeyer et al., 2017), which indicated the value of suspending fragments in the water column. Furthermore, fragments of coral on frames had slightly higher incremental growth rates, and the planar arrangement of the corals may have reduced competition for food among adjacent fragments because all fragments experienced more similar exposures to currents that carried food. In fact, previous work with trees indicated that higher densities of corals within a nursery restricted growth (O'Donnell et al., 2017).

Beyond enhanced growth rates, the planar structure of frames offered practical advantages, with divers reporting less incidental contact with fragments of coral and less interference when two divers worked on a single nursery (**Supplementary Video 1**). Such advantages should be particularly valuable to operations that employ less experienced divers because skilled divers could measure corals and volunteers could focus on maintenance without undue interference.

Additional support for the value of deploying frames and establishing nurseries in deeper water was provided by the effects of a severe winter storm that occurred a month after this study was completed. The storm dislodged two of the three trees and several large coral fragments from the remaining tree at the shallow site. In comparison, only one frame at the shallow site suffered minor damage and no fragments were lost or damaged. The frames included two points of attachment to the substrate and two floats, which appeared to provide redundancy and resistance to high energy events. Although frames proved beneficial, depth provided protection to both types of nurseries, with none of the structures at the deep site being affected even though it was only 100 m away from the shallow site.

Results from this study have informed practices undertaken by staff of the Central Caribbean Marine Institute on Little Cayman Island. By 2018, the shallow nursery had been retired, the deep nursery was expanded (**Supplementary Video 2**), additional deep nursery sites were planned, and trees were being replaced by frames. The quantitative and qualitative lessons from this work should be applicable to other efforts to grow and outplant coral fragments and to the more challenging tasks of culturing sufficient numbers of corals that will survive and thrive after outplanting (Rinkevich, 2014; Lirman and Schopmeyer, 2016).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PM and TF conceived the study. PM performed field and laboratory work and drafted the manuscript. HH contributed to field work. PM, CJ, and HH performed analyses and produced figures. PM, HH, CJ, and TF interpreted the data. CJ, HH, and TF contributed to revisions of the manuscript. TF contributed funding. All authors approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.670474/full#supplementary-material

Supplementary Figure 1 | Regressions of total linear extension on days since installation. Data for trees (gray squares) are offset by 1 day to allow data for frames (black circles) to be visible. All regressions are significant at $p < 0.001$. Statistics for regressions: Yellow genotype on frames at the shallow site: $y = 0.21x + 13.80$, $R^2 = 0.648$; yellow genotype on trees at the shallow site: $y = 0.16x + 12.20$, $R^2 = 0.643$; yellow genotype on frames at the deep site: $y = 0.26x + 9.87$, $R^2 = 0.822$; yellow genotype on trees at the deep site: $y = 0.25x + 9.78$, $R^2 = 0.784$; black genotype on frames at the shallow site: $y = 0.15x + 10.50$, $R^2 = 0.789$; black genotype on trees at the shallow site: $y = 0.14x + 11.48$, $R^2 = 0.659$; black genotype on frames at the deep site: $y = 0.22x + 9.47$, $R^2 = 0.818$; black genotype on trees at the deep site: $y = 0.21x + 9.46$, $R^2 = 0.817$; green genotype on frames at the shallow site: $y = 0.20x + 9.47$, $R^2 = 0.813$; green genotype on trees at the shallow site: $y = 0.13x + 10.23$, $R^2 = 0.638$; green genotype on frames at the deep site: $y = 0.22x + 7.95$, $R^2 = 0.758$; green genotype on trees at the deep site: $y = 0.20x + 6.75$, $R^2 = 0.776$; red genotype on frames at the shallow site: $y = 0.13x + 10.63$, $R^2 = 0.683$; red genotype on trees at the shallow site: $y = 0.13x + 12.62$, $R^2 = 0.673$; red genotype on frames at the deep site: $y = 0.18x + 11.23$, $R^2 = 0.749$; red genotype on trees at the deep site: $y = 0.16x + 13.33$, $R^2 = 0.692$; blue genotype on frames at the shallow site: $y = 0.08x + 5.99$, $R^2 = 0.760$; blue genotype on trees at the shallow site: $y = 0.11x + 11.92$, $R^2 = 0.388$; blue genotype on frames at the deep site: $y = 0.15x + 6.90$, $R^2 = 0.741$; blue genotype on trees at the deep site: $y = 0.17x + 10.60$, $R^2 = 0.747$.

Supplementary Figure 2 | Mean incremental growth rates ± standard errors (SE) for each replicate of each genotype suspended from the two nurseries at the two depths.

Supplementary Video 1 | Video of divers cleaning frame and trees at the deep site. Note that two divers can work simultaneously on the frame structure, while the tree is limited to a single diver.

Supplementary Video 2 | Video of the CCMI deep site expansion in 2018. New frames have been added and trees are being phased out. These structures contain over 500 linear meters of coral fragments available for outplanting.

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frontiers in Marine Science

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Approach to the Functional Importance of *Acropora cervicornis* in Outplanting Sites in the Dominican Republic

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Coral restoration has been recognized as an increasingly important tool for coral conservation in recent years. In the Caribbean, the endangered staghorn coral, *Acropora cervicornis* has been studied for restoration for over two decades with most studies focusing on evaluating simple metrics of success such as colony growth and survivorship in both nurseries and outplanted sites. However, for reef restoration to aid in the recovery of ecological function in outplanted sites, there is a need to measure the functional ecology of the impact of outplanting. Here, we present and identify positive ecological processes and ecological functions (such as increased fish biomass, coral cover, and increased in structural complexity) relative to active reef restoration. In the Southeastern Reefs Marine Sanctuary in the Dominican Republic, we monitored the percentage of benthic cover and fish biomass alongside active reef restoration over the period of 12 months in four zones. Subsequently, we developed multidimensional analyses in conjunction with generalized linear models (GLM) and linear models. Our results show there is a remarkable spatial and temporal differentiation favoring greater ecological function in restored areas. We observed the most noticeable patterns of change in the benthos and coral species composition. We found a positive relationship between amounts of outplanted colonies with the total fish biomass for the three outplanted sites. We highlight that *Scarus iseri*, a parrotfish critical for grazing maintenance, was the species with the greatest benefit. Our results provide evidence of the functional importance of *Acropora cervicornis* in coral reef active restoration efforts.

Keywords: *Acropora cervicornis*, coral reef restoration, outplanting sites, ecosystem services, Dominican Republic

INTRODUCTION

For decades, coral reefs have undergone a series of changes in structure and function due to a wide range of environmental and anthropogenic impacts (D'agata et al., 2014; Anthony et al., 2015; Pendleton et al., 2016; Hughes et al., 2017). Therefore, one of the challenges for researchers, authorities and local communities is to achieve the restoration of these ecosystems and their

services (Hughes et al., 2018; Lamb et al., 2018). Over the past 20 years, active reef restoration through human intervention has increased worldwide to mitigate the decline in coral cover. Propagation of corals for restoration is now considered an essential component of coral reef conservation and management strategies (Rinkevich, 2005, 2015; Precht, 2006; Edwards and Gómez, 2007; Petersen et al., 2007; Edwards, 2010; Johnson et al., 2011; Nakamura et al., 2011; Toh et al., 2012; Young et al., 2012; Chamberland et al., 2015; Lirman and Schopmeyer, 2016; Schopmeyer et al., 2017; Calle-Triviño et al., 2018, 2020; Bayraktarov et al., 2020; Shaver et al., 2020).

In the Caribbean, in the 1980s, there was a loss of up to 97% cover of *Acropora cervicornis* and *Acropora palmata* (Gladfelter, 1982; Porter et al., 1982; Knowlton, 1992; Miller et al., 2002), this decline caused its inclusion in the Union for Conservation of Nature (IUCN) as critically endangered species, and in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Aronson et al., 2008). It decreases also has resulted in losses in the three-dimensional structure of shallow reefs and in ecological, economic, and social services (Bruckner, 2002; Vargas-Ángel et al., 2006; Alvarez-Filip et al., 2009).

A. cervicornis has had limited recovery due to the interactions and positive feedback of natural and anthropogenic stresses that exist at both local and global levels (Precht et al., 2002; Weil et al., 2002; Carpenter et al., 2008; Agudo-Adriani et al., 2016). To promote coral cover recovery, restoration programs in the Caribbean region have intensified the propagation of *A. cervicornis* fragments (Bowden-Kerby, 2001; Hernández-Delgado et al., 2001; Young et al., 2012; Lirman et al., 2014). While restoration efforts have increased exponentially (Lirman and Schopmeyer, 2016), few studies have been published on the recovery of ecosystem functions and services in outplanting sites (Griffin et al., 2012; Lirman et al., 2014; Schopmeyer et al., 2017; Calle-Triviño et al., 2020). The branching morphology of *A. cervicornis* provides important structural complexity for different reef organisms. Complex interactions and energy flows are formed around this species, such as high levels of primary productivity and associations between different species (Itzkowitz, 1978; Lirman, 1999; Bruckner, 2002; Goergen et al., 2019). Because of its life history characteristics and its high growth rate it has been one of the species selected to develop restoration projects in the Caribbean (Young et al., 2012; Calle-Triviño et al., 2018). However, no published scientific studies address functional aspects of *A. cervicornis* in outplanted areas, and a deeper understanding of the role this species plays in creating and modifying reef fish habitats is needed (Huntington et al., 2017).

Generally, the "success" of restoration programs in the Caribbean region has been measured a single variable (i.e., growth, survival, annual productivity or percentage coverage) (Lirman and Schopmeyer, 2016; Schopmeyer et al., 2017; Ladd et al., 2018, 2019; Calle-Triviño et al., 2020; Seraphim et al., 2020). This single-variable approach at the organism level does not allow for the identification of successional processes that occur in outplanted areas and the effects on functions and resilience in these areas are unknown. The scale of the analysis to the ecosystem level to describe correlations among groups can be useful tool to evaluate restoration programs, on the premise that *A. cervicornis* performs as an indirect facilitating agent, providing three-dimensionality across habitat, increasing refuge availability, niches, food availability and regulating interactions between organisms on coral reefs (Graham and Nash, 2013; Agudo-Adriani et al., 2016; Floros and Schleyer, 2017).

In 2011, the Dominican Foundation for Marine Studies (FUNDEMAR) began its coral restoration program with the purpose of using fragments of the *A. cervicornis* coral to attempt to repopulate degraded reef areas in Bayahibe on the southeastern end of the island (Calle-Triviño et al., 2020).

In 2015, three outplanted sites and one control site were monitored for twelve consecutive months to identify if there was an influence on benthic composition, abundance of coral and fish species in the outplanted sites over time, and the interaction between these variables. In this study, we analyzed changes in benthos and in fish communities due to restoration actions in outplanted sites in the Southeaster Reefs Marine Sanctuary in the Dominican Republic.

MATERIALS AND METHODS

Study Location

The outplanted sites studied are part of the Southeast Reefs Marine Sanctuary (**Figure 1**). The sanctuary includes a chain of coral reefs located along the southeastern coast of the Dominican Republic with a total area of 7,862.59 km^2. It was declared a protected area on August 7, 2009 by Decree No. 571-09. The reefs within the sanctuary and adjacent areas are an important tourist attraction. The study was carried out in three outplanted sites ZT1, ZT2, ZT3, and a control site "Peñon" (**Table 1**) all included in the area of the municipality of Bayahíbe.

Outplant and Control Site Establishment

During the selection and establishment of the outplanting and control sites, prospective dives were carried out in order to ensure that the selection criteria were achieved. Criteria for selection of outplanting sites included: depth (between 12 and 15 m), presence of wild *A. cervicornis* colonies, low sedimentation, low macroalgae cover, and the presence of calcareous coralline algae (CCA) (Edwards, 2010; Johnson et al., 2011; Mercado-Molina et al., 2013; Arias-González et al., 2015; Carne et al., 2016). Before outplanting, the substrate was cleaned using different hand tools (brushes, chisels, hammers) to remove algal mats, sediments, or macroalgae, but the CCA was undisturbed (Calle-Triviño et al., 2020).

After properly preparing the substrate, squared galvanized masonry steel nails were placed in the substrate, keeping a distance of 0.5–1 m between the nails (Mercado-Molina et al., 2013) obtaining outplant densities of 1.5 colonies per square meter. All of the outplanted colonies were harvested from FUNDEMAR's main nursery. At that time, the genotype of the fragments was not taken into account, since the nursery had not yet been genotyped. However, it is currently known the nursery supports at least 32 individuals, indicating there was

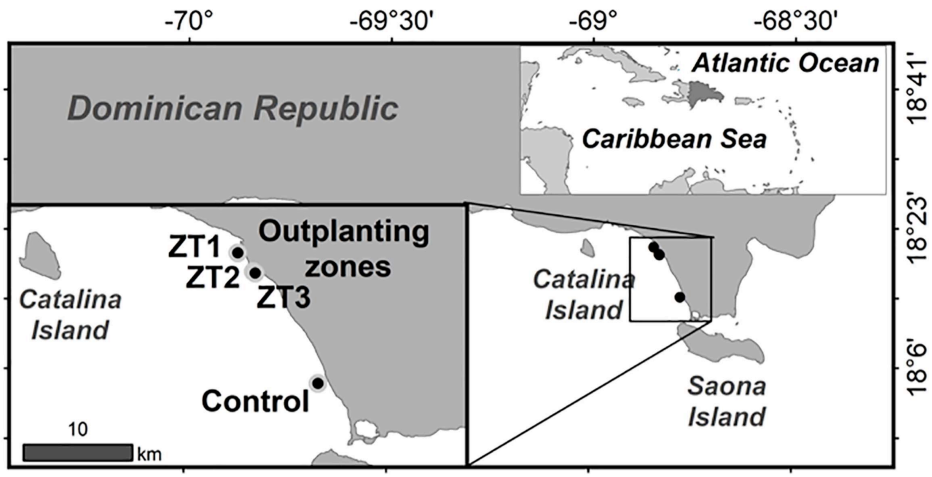

FIGURE 1 | Map of outplanted sites and control site part of the Arrecifes del Sureste Marine Sanctuary.

likely a mixture of genets that contributed to the outplanting. All the outplanted colonies had a size > 45 cm of linear growth, and fixed to the pre-established nails with plastic cable ties (Johnson et al., 2011). All the selection criteria and the techniques used to perform the transplants are described in Calle-Triviño et al. (2020). In each of the outplanting sites, a total of 200 colonies were outplanted in an area of 200 m^2, at a depth between 12 and 15 m.

The control area (Peñon) was chosen taking into account different selection criteria, including depth (which was similar to the outplanting areas, between 12 and 15 m), distance from outplant sites (<500 m), and available historical information (Cortés-Useche et al., 2019). A single control site was chosen due to logistical and budgetary limitations of the project.

Sampling Design

In each of the three outplanted sites and in the control site, six transects of 10 m each were randomly selected based on the AGRRA (Atlantic and Gulf Reef Rapid Reef Assessment) Version 5.4 protocol (Lang et al., 2010). These transects were subsequently installed permanently along the sites to carry out the assessments. Four monitoring cycles were conducted during a 12-month period. The point intercept methodology was used for the benthos information survey, with measurements collected every 10 cm in each of the six 10 m transects; the category corresponding to the substrate observed just below each point was recorded. To determine the abundances of fish in each of the outplanted areas and the control area, four temporary belt transect surveys were performed (30 m long × 2 m wide) in the same habitat as the permanent transects. In each belt transect, the number of individuals corresponding to the commercially and/or ecologically important reef fish species covered by the AGRRA protocol was recorded, as well as their sizes in the proposed class size ranges in the protocol. Using the abundance and size class data, biomass was calculated using the length-weight relationship equation $W = aLb$ described by Bonsack and Harper (1988). Constants (a and b) for length-weight relationships for each species were obtained from Froese and Pauly (2019).

TABLE 1 | Codes and Geographical coordinate of the outplanted sites and control site in Bayahíbe, Dominican Republic.

	Geographic coordinate	
Codes	**N**	**E**
ZT1	18.3609°	−68.84515°
ZT2	18.34533°	−68.83232°
ZT3	18.34424°	−68.83087°
Control	18.253°	−68.779°

In addition, we used the methodology proposed by Schopmeyer et al. (2017) to evaluate the growth, survival, and productivity of the colonies transplanted in outplanting sites. Schopmeyer et al. (2017) proposed the following reference points for measuring the first year of *A. cervicornis* restoration: (1) the survival of outplanted corals must be greater than 70% and (2) average productivity should be > 4.8 cm $year^{-1}$ for outplanted corals.

We monitored sites every 3 months during the 12-month period after their establishment. Colonies from outplanted sites were individually labeled. Growth was expressed as Total Linear Extension (TLE) in cm over time for each coral, measuring from base to tip of each branch and adding up all the branches to obtain total growth of each colony (Johnson et al., 2011; Lirman et al., 2014). Data on growth, survival, and annual productivity were taken as presented by Calle-Triviño et al. (2020), and proposed and used by Lirman et al. (2014) and Schopmeyer et al. (2017) as follows:

- Total annual growth = (Final Measure − Initial Measure).
- %Survival = (# final live colonies × 100)/# initial live colonies.
- Annual productivity = (growth/initial TLE).

The mean annual productivity was calculated by pooling all outplants. This measure was proposed by Lirman et al. (2014) and used by Schopmeyer et al. (2017). We have used the same measure in order to compare results in this publication with similar studies in the United States and Puerto Rico.

Benthos Analysis and Coral Species Composition

Generalized linear model (GLM) analysis was applied to identify changes in composition of benthos, coral, and fish species in the outplanting sites over time. This analysis considers variations in abundance values and allows us to consider different types of error distributions (Warton et al., 2015). For the GLMs the negative binomial distribution was used with a link function of logarithm, because it showed the best results, reducing the over-dispersion present in the data. For hypothesis testing, 999 permutations were applied using Monte Carlo simulations, and considered within the univariate analysis influence (significance of each species) for which an adjustment procedure based on multiple tests from step-by-step resampling was used. This analysis was performed using the "mvabund" library of the statistical program R (Warton et al., 2012; R Development Core Team, 2015).

In order to know the fit and confirm the model assumptions were not violated, analysis graphs were obtained for model residuals. In other work conducted on coral reefs, biomass has been properly modeled from negative binomial GLM, thus capturing over-dispersion present in the biomass data (Ferrari et al., 2018). The ordination was visualized from a non-metric multidimensional scaling, based on the Bray-Curtis dissimilarity matrices, using data transformed to the logarithm. This analysis was made from program R's "vegan" library. These analyses were conducted for all data transects within each site and on each of the dates sampled.

Measuring Ecological Indicators Associated With Outplanting Efforts

To determine the increase in some variables, considered as a positive effect due to outplanting actions, two main variables known as coral indicators were calculated and obtained: (1) coral cover and (2) coefficient of functionality. Moreover, the total fish biomass and biomass of species showed a significant temporal change in the analysis of GLM.

Coral coverage was obtained directly from the benthos percentage coverage data. The coefficient of functionality was calculated considering the values and equation presented in the work of González-Barrios and Álvarez-Filip (2018). This is derived from the Reef Functional Index (RFI) which is a site-level indicator. The coefficient within the RFI quantifies the structural complexity of coral based on parametric models of coral growth and complexity of morphology (González-Barrios and Álvarez-Filip, 2018). We decided to explore the use of combined descriptors such as the RFI, as an additional descriptor to the analysis that may be relevant in reef research, provided that the robustness of this index is demonstrated in future work.

TABLE 2 | Coral and fish species observed in the three outplanting sites and control sites.

Coral species	**ZT1**	**ZT2**	**ZT3**	**CONTROL**
Acropora cervicornis	X	X	X	X
Agaricia spp.	X	X	X	X
Colpophyllia natans	X	X	X	X
Dendrogyra cylindrus	X	X		
Dichocoenia stokesii				X
Diploria labyrinthiformis	X	X	X	X
Eusmillia fastigiata	X			X
Favia fragum			X	
Isophyllia sinuosa	X			
Madracis spp.	X	X	X	X
Meandrina meandrites	X	X	X	X
Millepora alcicornis	X	X	X	X
Montastraea cavernosa	X	X	X	X
Mussa angulosa				X
Mycetophyllia spp.		X	X	X
Orbicella annularis	X	X	X	
Orbicella faveolata	X	X	X	X
Orbicella franksii	X	X	X	X
Porites astreoides	X	X	X	X
Porites divaricata	X			X
Porites furcata	X			X
Porites porites	X	X	X	X
Pseudodiploria clivosa	X			X
Pseudodiploria strigosa	X	X	X	X
Scolymia spp.				X
Siderastrea radians				X
Siderastrea siderea	X	X		X
Solenastrea bournoni	X	X		X
Stephanocoenia intersepta	X			X
Fish species	**ZT1**	**ZT2**	**ZT3**	**CONTROL**
Acanthurus bahianus	X	X	X	X
Acanthurus chirurgus	X	X	X	X
Acanthurus coeruleus	X	X	X	X
Aluterus scriptus	X	X	X	X
Balistes vetula	X	X	X	X
Bodianus rufus	X	X	X	X
Cantherhines macroceros	X	X	X	X
Cantherhines pullus	X	X	X	X
Caranx ruber	X	X	X	X
Chaetodon aculeatus	X	X	X	X
Chaetodon capistratus	X	X	X	X
Chaetodon ocellatus	X	X	X	X
Diodon hystrix	X	X	X	X
Epinephelus cruentatus	X	X	X	X
Epinephelus fulvus	X	X	X	X
Haemulon aurolineatum	X	X	X	X
Haemulon carbonarium	X	X	X	X
Haemulon chrysargyreum	X	X	X	X
Haemulon flavolineatum	X	X	X	X
Haemulon plumieri	X	X	X	X
Haemulon sciurus	X	X	X	X
Halichoeres garnoti	X	X	X	X

(Continued)

TABLE 2 | Continued

Coral species	ZT1	ZT2	ZT3	CONTROL
Holacanthus tricolor	X	X	X	X
Lactophrys bicaudalis	X	X	X	X
Lutjanus analis	X	X	X	X
Lutjanus apodus	X	X	X	X
Lutjanus mahogoni	X	X	X	X
Lutjanus synagris	X	X	X	X
Melichthys niger	X	X	X	X
Microspathodon chrysurus	X	X	X	X
Ocyurus chrysurus	X	X	X	X
Pomacanthus paru	X	X	X	X
Pterois spp.	X	X	X	X
Scarus iseri	X	X	X	X
Scarus taeniopterus	X	X	X	X
Scarus vetula	X	X	X	X
Sparisoma atomarium	X	X	X	X
Sparisoma aurofrenatum	X	X	X	X
Sparisoma chrysopterum	X	X	X	X
Sparisoma viride	X	X	X	X
Sphoeroides spengleri	X	X	X	X

Regression models were performed to explain changes in cover, RFI, Total fish biomass and *Scarus iseri* biomass as a function of time (as an indicator of effort from outplanting actions), as well as models explaining changes in total fish biomass and significant fish species in the multi-dimensional GLM as a function of coral cover percentage and change in RFI (as a measure of structural complexity and proxy indicator of coral cover). Linear regression models, based on median data for sampling site and date, were chosen for this analysis using the untransformed data for coral cover and RFI, as well as a log transformation for fish biomass. Graphical residual assessments and a global test of linear models were carried out to verify the regression models' assumptions (Pena and Slate, 2006). Regression curves of the prediction lines and 95% Wald confidence bands were plotted using the "visreg" package of the R program (Breheny and Burchett, 2017).

Hurricane Season 2016 and 2017

The impact of Hurricanes Mathew (2016), Irma and Maria (2017) could only be quantified for benthos in ZT1, due to the climatic and logistical conditions occurring in the area just after those events.

To recognize differences in coral cover and RFI, as well as total fish biomass during the period of the hurricanes (September 2016 and September 2017), a strong statistical paired sample test was conducted. In comparison, the values for the indicators mentioned above were used at the site level, considering as dependent samples each of the transects carried out during September 2016 and February 2017. There was no homogeneity of variances or normality, so we chose to perform a Yuend test for difference in trimmed means, considering only values found within the 10th and 80th percentiles of the data distribution. For the test, a 95% confidence level was considered, based on the WRS2 library of the R statistical program (Mair et al., 2016).

RESULTS

In the three outplanting and control sites, 29 species of coral and 41 species of fishes were observed (**Table 2**). The Scaridae family presented highest abundances, followed by the Acanthuridae family. During the 12 months of study, changes in benthic coverage (**Supplementary Table 1**), relative coverage of coral species (**Supplementary Table 2**), and relative biomass of fish species (**Supplementary Table 3**) were observed across sites, dates, and site-date interactions.

Growth, Survival and Annual Productivity

Mean survival of *A. cervicornis* fragments for the three outplanted sites during the 12-month period was 67.16 ± 13.8%, with a range of 57–83%. During this period, the most common cause of mortality was sedimentation and predation by the fireworm, *Hermodice carunculata* (Calle-Triviño et al., 2017, 2020). The three outplanted sites' mean productivity value was 3.53 ± 1.40 cm year^{-1} (**Table 3**).

Analysis of Change in Composition: Benthos, Coral, and Fish Species

Species composition showed a marked differentiation among the study sites considered. This variation was significant for benthic coverage, as well as in coral and fish species. Similarly, the variation in species composition over time was significant in benthic groups, corals, and fish species. The difference in species composition is greater only between zones or only between dates. Within each site between dates there was not statistically significant (**Table 4** and **Figure 2**).

For benthic coverage composition, all components of the benthos were identified as important contributors between zones, being the abiotic substrate the only coverage value that was not significant. Outplanting sites ZT2 and ZT3 are very similar, while site ZT1 shows stronger differentiation, especially

TABLE 3 | Annual productivity and survival of *Acropora cervicornis* fragments in the three outplanted sites.

	ZT1	ZT2	ZT3
Annual productivity	4.9	3.6	2.1
Survival (%)	83	61	57.5

TABLE 4 | Variation in species composition between dates, zones and between dates and zones.

	Benthos cover GLM = 14.67 *p*-value = 0.001	Coral species GLM = 21.42 *p*-value = 0.001	Fish species GLM = 24.41 *p*-value = 0.001
Zone	161.06 (0.001, 92)	472.3 (0.001, 92)	381.6 (0.001, 60)
Date	38.40 (0.001, 91)	109.5 (0.001, 91)	61.0 (0.001, 59)
Zone: date	37.74 (0.049, 88)	51.7 (0.081, 88)	67.2 (0.062, 56)

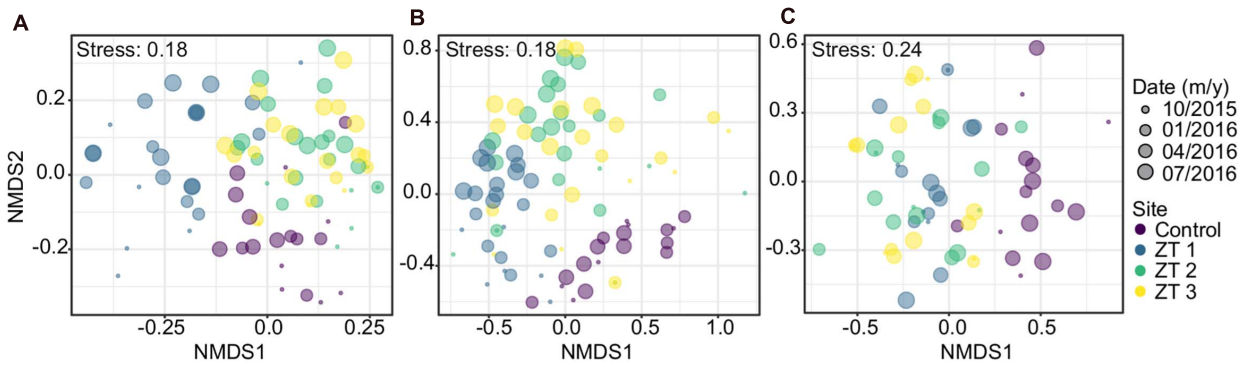

FIGURE 2 | Non-metric multidimensional scaling (NMDS) analysis based on groups' composition: **(A)** benthos, **(B)** corals, and **(C)** fishes in the three outplanted sites and the control site over time.

at earlier dates. Throughout time, outplant sites tended to be increasingly similar in their composition (**Figure 2**). Change in benthic cover composition over time was mainly attributed to increase coral cover, decrease macroalgae, and abiotic substrate in outplanted areas, these types of cover being significant in the temporal change.

Moreover, 13 of the 29 registered coral species were found to contributed significantly to the variation between areas. The most noticeable result in the case of the temporal variation in the composition of coral species, those that presented significant values, were the species *A. cervicornis* and those belonging to the genus *Agaricia* spp., observing a general increase of the former (because of the outplanting) and a decrease of the latter (**Supplementary Table 2**).

Regarding differentiation in fish species composition between zones, six of the 41 species registered was significant (**Supplementary Table 3**). In fish species the only significant temporal variation was the species *S. iseri*, which presented a considerable increase especially in the outplanted sites.

Ecological Benefits Due to Outplanting

Analysis of the regression models showed clear ecological benefits due to transplantation, mainly expressed in increased coral cover and increased structural complexity evidenced in the RFI (**Figures 3A,B**). If we consider date as a descriptive variable in the linear models (**Figures 3C,D**), the fitting (R^2) is very low and not significant (**Table 5**). However, this increase in habitat complexity and coral cover also reflected in an increase in total biomass for all fish species sampled, especially *S. iseri* (**Figures 3E–H**).

Coral coverage showed an annual increase of 24.69% ± 5.40% SE in the outplanted sites. This increase in coral cover was directly reflected in an increase in RFI, showing an increase of 0.141 RFI, ± 0.03 SE per year (RFI theoretically ranges from a scale of 0 to ∼1), mainly due to the contribution of *A. cervicornis*, a species of high functional value (**Table 5**). In general, it was observed that the ZT1 area presented a lower increase of coral cover, since from the beginning of outplanting events, it was the one that presented the highest values for coral cover (**Figures 3A,B**).

The relationship between RFI and coral cover with fish biomass showed an exponential increase, expressed from a linear model with a logarithmic transformation of fish biomass (**Figures 3E–H** and **Table 5**). The increase of ∼20% of coral cover and 0.15 RFI (∼the 1-year increase in transplant effort) is reflected in an increase of ∼1,100 g/100 m^2 in untransformed values of fish biomass. This increase in biomass was also found in the species *S. iseri* (the only species found to be significantly P permuted < 0.05 in the multivariate GLM). The increase in *S. iseri* biomass was also exponential related to the RFI and coral cover increase, showing a slightly better adjustment than the total biomass (**Table 5**) and an increase of ∼200 g/100 m^2 in untransformed values of biomass due to an increase of ∼20% of coral cover and 0.15 RFI.

While efforts in the outplanting sites were intensified, RFI increased directly proportional to the increase in percentage of coral coverage. At the same time, the percentage of macroalgae coverage and abiotic substrate available for colonization decreased.

Ecological Costs Due to the 2016 and 2017 Hurricane Season

During the study period, three hurricanes (Hurricane Matthew, 2016; Irma and Maria, 2017) directly impacted outplanted sites and some reefs in the Southeast region of the Dominican Republic (National Hurricane Center, NOAA). The impacts of these hurricanes were reflected in the outplanted sites, finding change in species composition and a significant loss of coverage of 24% for the ZT1 outplanted site (the only one in which the benthos following the impact of hurricanes could be quantified), which occurred between September 2016 and February 1, 2017 (**Figures 4A,B**). This loss of coverage resulted in a decrease of 0.103 RFI. These results show a significant reduction caused by the Hurricane season (**Table 6**).

Ecological cost due hurricanes on the fish biomass was not so evident, finding a significant decrease of the total biomass of 1,874.1 g/100 m^2 only in the ZT1 site (p-value = 0.030). However, in other outplanted areas, a decrease in biomass was also identified, despite not being statistically significant (**Table 6**).

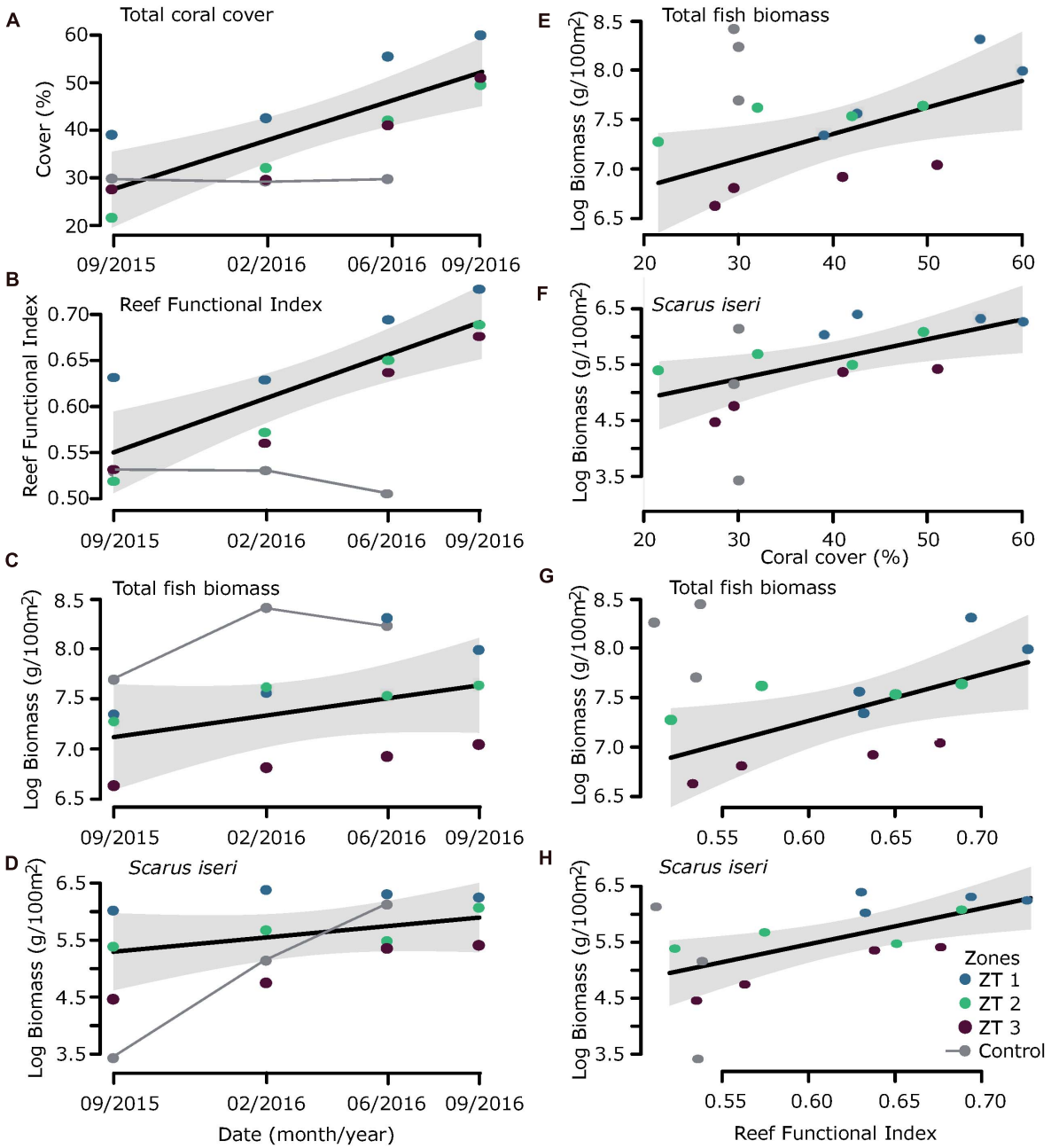

FIGURE 3 | Ecological benefits due to outplanting. Considering date as a descriptive variable **(A)** coral cover, **(B)** Reef Functional Index (RFI), **(C)** total fish biomass, and **(D)** *Scarus iseri* biomass. considering Coral cover and RFI as a proxy of habitat complexity, reflected increase in **(E,G)** total fish biomass and **(F,H)** *Scarus iseri* biomass.

DISCUSSION

To our knowledge, this is the first study to explore the restoration of ecological functions at *A. cervicornis* outplanted sites in the Dominican Republic by including approaches using multiple variables to describe the correlations between the habitats studied (Bayraktarov et al., 2020; Boström-Einarsson et al., 2020; Seraphim et al., 2020). Here, we demonstrate that active restoration efforts result in direct ecological benefits, and help restore ecological function, expressed in the increased coral cover, structural complexity, and fish biomass described in this study.

To understand the relationship between physical structure features and associated fauna, we monitored progression of two habitat descriptors, both structural, over 12 months, and their association with variation in fish abundance, to identify the role

TABLE 5 | Linear models of the different variables used during 12-months period of study.

Linear model	DF	*F*-statistic	R^2	*p*-value
Coral cover ~ Date	10	20.89	0.6763	0.001025
RFI ~ Date	10	22.07	0.6882	0.0008446
Biomass ~ Date	10	2.077	0.172	0.1801
Scarus iseri ~ Date	10	1.697	0.1451	0.2219
Biomass ~ Coral cover	10	7.139	0.4165	0.02342
Scarus iseri ~ Coral cover	10	8.477	0.4588	0.01552
Biomass ~ RFI	10			
Scarus iseri ~ RFI	10	9.502	0.4872	0.01159

TABLE 6 | Ecological cost due hurricanes in three outplanting sites and one control.

	Zone	Adjusted mean difference (confidence intervals)	*p*-value	Size effect
Coral cover	ZT1	−24 (−33.894 to −14.106)	0.005*	0.91
Coral functional coefficient	ZT1	−0.103 (−0.166 to −0.04)	0.0134*	0.93
Fish biomass	ZT1	−1,874.1 (−3,399.1 to −349.2)	0.030*	0.52
	ZT2	−359.8 (−3,015.8 to 2,296.2)	0.695	0.21
	ZT3	−293.7 (−803.5 to 216.1)	0.164	0.41
	Control	−141.1 (−2,316.7 to 2,034.6)	0.8497	0.08

*$P < 0.05$.

that *Acropora* spp. has in maintaining ecosystem services, not only as a creation of breeding habitats for fish (Darling et al., 2017; Floros and Schleyer, 2017), but also by decreasing the space available for colonization by opportunistic fast-growing organisms, such as algae and/or sponges (Agudo-Adriani et al., 2016; Mora et al., 2016). Furthermore, as in this case, the change in the relative cover between *A. cervicornis* and *Agaricia* spp. can be beneficial, considering that our results indicate that with restoration efforts, a change in the increased dominance of *A. cervicornis* is obtained, which would help to recover previous reef states (O'Dea et al., 2020), as efforts in the outplanting sites increase.

Few studies have tracked the community dynamics of reef organisms after restoration (Opel et al., 2017; Bayraktarov et al., 2020; Boström-Einarsson et al., 2020; Seraphim et al., 2020). The results presented in this study show that the positive effects of restoration are reflected in a temporal variation in benthic cover composition, as well as in coral and fish species composition. They show that with the rehabilitation of a single species (*A. cervicornis*), the functions of an outplanted site, such as herbivory, are recovered by increasing the total biomass of fish and in particular of the parrotfish *S. iseri*. In this case although this is a small-scale study we were able to observe positive changes over time while active restoration actions were constantly carried out in these reef patches that are part of an important MPA for the southeastern zone of the country (Shaver and Silliman, 2017; Calle-Triviño et al., 2018, 2020; Cortés-Useche et al., 2018, 2019, 2021).

Increased *A. cervicornis* coverage may improve the functions of coral reef ecosystems by generating beneficial interactions between species (Shaver and Silliman, 2017), as for example in this case study where it was evident that by performing constant actions of active restoration, such as removal macroalgae when preparing substrate in outplanting sites and by having the surfaces occupied by colonies of *A. cervicornis,* the abiotic substrate available to be colonized also decreased, which may influence the decrease in the cover of opportunistic species such as sponges, algae mats, macro-algae that can contribute to increased bio-erosion (Yap, 2013). In addition, was increasing structural complexity, which was shown to increase RFI and total fish biomass in the outplanted sites.

Improved *A. cervicornis* cover provides increased structural complexity and architecture of the ecosystem which, in turn provides a greater number of refuges and feeding grounds for other commercially and/or ecologically important invertebrates

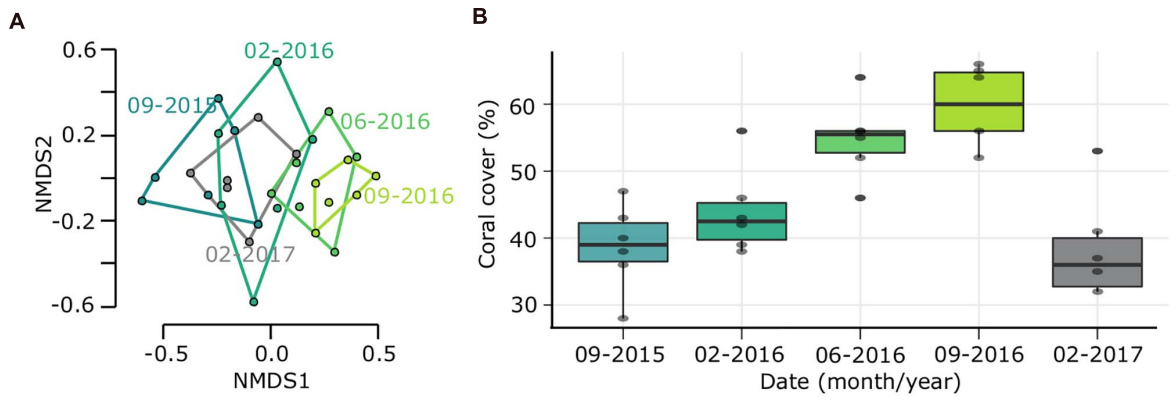

FIGURE 4 | Ecological costs due to Hurricane season 2016-2017. **(A)** Non-metric multidimensional scaling (NMDS) analysis based on species composition in outplanted site ZT1 over time. **(B)** Change in coral cover over time.

(e.g., octopus, lobsters) and of course for reef fish (Cabaitan et al., 2008; Yap, 2009; Schopmeyer and Lirman, 2015; Huntington et al., 2017; Opel et al., 2017; Shaver and Silliman, 2017). Thus, observation of the dynamics of fish communities after transplants is fundamental to understanding the ecology of the system and evaluating the "rapid" contributions of restored sites (Opel et al., 2017; Ladd et al., 2019; Seraphim et al., 2020). Some authors report that outplanting efforts in coral restoration projects cause an immediate change in the ecological function and services of degraded regions of the reef, therefore, they think that restoration influences and in turn facilitates the repair of ecosystem function (Agudo-Adriani et al., 2016; Lirman and Schopmeyer, 2016; Opel et al., 2017; Shaver and Silliman, 2017). However, of the more than 200 cases on coral restoration in published scientific literature, whose objectives were primarily aimed at assessing recovery of ecosystem functions, appropriate metrics were not used to assess project success in relation to that objective (Boström-Einarsson et al., 2020).

In the case of fishes, although the temporal variation is significant, it is not as strong as the two components mentioned above (coral cover and structural complexity), in this variation there is a considerable increase in the *S. iseri* species. We therefore suppose that this particular species has a strong association with *A. cervicornis*. Nevertheless, this is only the first result derived from a small-scale experiment. It is worth mentioning that *S. iseri* is a species that benefits when there is a high connection between habitats, as in the case of mangroves, sea grasses and coral reefs (Mumby et al., 2004; Harborne and Mumby, 2018), these three ecosystems are present in the study area within < 1 km distance from each other. The presence of these three together may contribute to the recruitment of *S. iseri*. Our results suggest that *A. cervicornis* may be an optimal habitat that can facilitate the reintroduction of *S. iseri*, and it is likely that this fish species is a good indicator of improved fish recruitment conditions as a function of habitat improvement. Considering that this fish is associated with highly complex reefs, it is probable that increasing RFI will be reflected in increased *S. iseri* due to increased habitat availability.

Our results suggest that through active restoration, positive outcomes in reef health are seen. Over short periods of time, this recovery does not equate to sustained recovery of the ecosystem, particularly since events such as El Niño, warming events (which can associate pathogenic microorganisms and disease outbreaks), increased acidification, among others, are becoming more frequent thus reducing recovery time (Hughes et al., 2018; Goergen et al., 2019). The assessment conducted here indicates that after the hurricanes, although there was a decrease in fish biomass, the loss would probably have been greater without restoration efforts (**Figure 5**). It would be important to observe these analyses when transplanting with other reef-building species such as those of the *Orbicella* complex, which have different functional traits, and to use different indicators that can provide a proxy for the changes that can be observed at the ecosystem level.

These results offer a hopeful glimpse into the return of certain ecosystem functions with sustained reef restoration of *A. cervicornis*. It will be necessary to monitor the changing ecology of restoration projects long-term to further validate the longevity of these results. While the standard biological metrics of restoration efforts should continue (such as survival and growth of the transplanted corals), this study indicates the functional ecology must also be monitored (Goergen et al., 2020).

This study represents an important advance in the restoration of Caribbean coral reefs, which have been exposed to various impacts that have resulted in significant losses in their coverage, structural complexity and considerable loss of fish biomass (Gardner et al., 2003; Arias-González et al., 2017). Besides, it highlights the importance of restoration and active conservation measures for coral reefs.

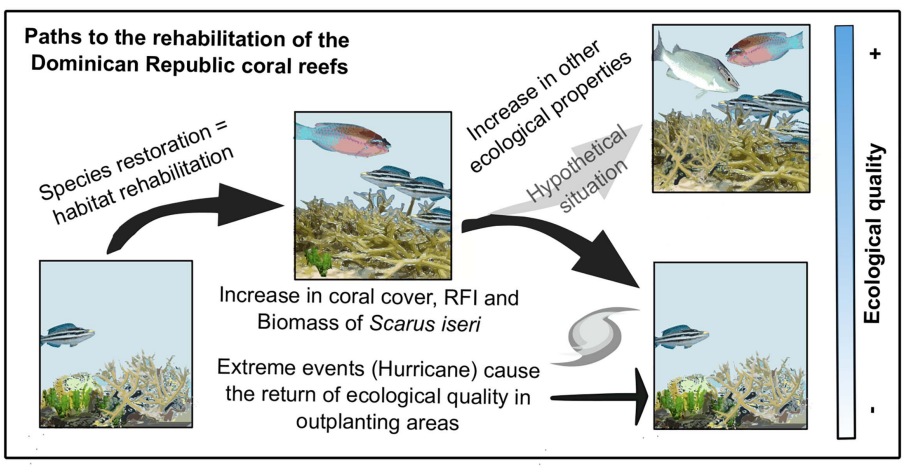

FIGURE 5 | Conceptual model. Species restoration induces habitat rehabilitation, increasing coral cover, RFI, and biomass in this particular case study of *Scarus iseri*. Damage from storms or hurricanes would be even greater if no active restoration efforts were undertaken. Continued active restoration efforts will improve the ecological health of coral reefs.

This study emphasizes the relevance of using ecosystem-level scales of analysis to describe correlations between fish communities and habitat to identify important changes in coral reef function and resilience (Graham and Nash, 2013), and their potential positive effect on coral reef systems. Furthermore, the importance of continuing to increase scientifically based active restoration efforts in the southeast region of the Dominican Republic and to continue to demonstrate the effectiveness of restoration within MPAs.

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 Ministerio de Medio Ambiente y Recursos Naturales de la República Dominicana.

AUTHOR CONTRIBUTIONS

JC-T conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the manuscript, and approved the final draft. AM-C analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft. CC-U performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft. MM prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft. RS-B performed the experiments and approved the final draft. JA-G conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.668325/full#supplementary-material

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Integrating Coral Restoration Data With a Novel Coral Sample Registry

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In the past decade, the field of coral reef restoration has experienced a proliferation of data detailing the source, genetics, and performance of coral strains used in research and restoration. Resource managers track the multitude of permits, species, restoration locations, and performance across multiple stakeholders while researchers generate large data sets and data pipelines detailing the genetic, genomic, and phenotypic variants of corals. Restoration practitioners, in turn, maintain records on fragment collection, genet performance, outplanting location and survivorship. While each data set is important in its own right, collectively they can provide deeper insights into coral biology and better guide coral restoration endeavors – unfortunately, current data sets are siloed with limited ability to cross-mine information for deeper insights and hypothesis testing. Herein we present the Coral Sample Registry (CSR), an online resource that establishes the first step in integrating diverse coral restoration data sets. Developed in collaboration with academia, management agencies, and restoration practitioners in the South Florida area, the CSR centralizes information on sample collection events by issuing a unique accession number to each entry. Accession numbers can then be incorporated into existing and future data structures. Each accession number is unique and corresponds to a specific collection event of coral tissue, whether for research, archiving, or restoration purposes. As such the accession number can serve as the key to unlock the diversity of information related to that sample's provenance and characteristics across any and all data structures that include the accession number field. The CSR is open-source and freely available to users, designed to be suitable for all coral species in all geographic regions. Our goal is that this resource will be adopted by researchers, restoration practitioners, and managers to efficiently track coral samples through all data structures and thus enable the unlocking of a broader array of insights.

Keywords: coral reefs, database, registry, coral restoration, coral collecting, accession number, Coral Sample Registry, restoration data

INTRODUCTION

The rapid decline in coral cover and health around the world is due to local, regional, and global threats (Hughes et al., 2018). The factors responsible for coral reef decline include climate change impacts (Hoegh-Guldberg et al., 2007; Carpenter et al., 2008; Doney et al., 2012) that cause coral bleaching and mortality (Hoegh-Guldberg, 1999; Eakin et al., 2010) and coral diseases (Aronson and Precht, 2001; Bruno et al., 2007). Complex interactions among herbivores, specifically fishes and urchins, seaweeds, and corals also impact the condition of coral reefs (Hixon, 2015). Proximity to large human populations is related to decline, where development, pollution, and overfishing can impact coral reef habitats (Hughes and Connell, 1999; Fabricius, 2005; Pendleton et al., 2016). Without substantial course alterations these stressors are expected to continue unabated, further degrading tropical coral reefs. This outcome would mean catastrophic loss of marine species, potential loss of tropical coral reef ecosystems, reduced food security for a large portion of the world's population, international security issues, risks to fresh water supplies, and increased coastal flooding. Consequently, protecting and restoring the world's tropical coral reefs has become increasingly important to both public and private interests across the global community broadly (Hein et al., 2021).

To successfully address the long-term stability of coral reef ecosystems, three courses of action are required: first, mitigation of the stressors leading to coral mortality; second, maintaining and expanding the current populations of reef-building corals; and third, implementing methods to help corals adapt to evolving environmental conditions (Duarte et al., 2020; Hein et al., 2020; Vardi et al. in review). Tackling each of these is a major undertaking requiring a multi-disciplinary approach and extensive coordination between research, resource management, and restoration agencies. Consequently, informal knowledge sharing organizations have been formed, such as the Coral Restoration Consortium (2021; Vardi et al. in review), the International Coral Reef Initiative (2021), and other large-scale, centrally coordinated projects (e.g., Reef Plan 2050, Australia; Mission: Iconic Reefs, FL, United States; Reefense, United States).

Coral restoration, defined here as active interventions including coral population management, propagation, outplanting, and research, drives specific courses of action to counteract threats and maintain and expand coral populations. This broad field is the product of integrating across resource management agencies, academic research groups, and restoration practitioners, each with distinct yet partially overlapping interests (**Figure 1**). Resource management agencies, charged with the protection and regulation of coral species and their environs, coordinate, permit, and track activities relative to an overall management plan in accordance with the policies of their sovereignty. They are concerned with the collection from wild colonies, properties of wild colonies (e.g., disease presence), distribution records, utilization of collected samples, survivorship, and so forth. Academic researchers inform specific aspects of the genomics, physiology, population structure and ecology of specific corals (species or strains), reef community interactions, or geographic regions. They are increasingly investigating the physiological and genomic mechanisms that give rise to differences in phenotypic response based on genetics and genome by environment interactions, a field that is increasing as identification of factors for resiliency become more important. Restoration practitioners are actively collecting, growing, and transplanting corals to degraded reefs. They track the quantities and relative performance of corals across nursery and outplant settings. For each of these groups, the tracking of individual samples and the corresponding data are critically important. With increases in restoration activities and research, the amount of data generated is rapidly proliferating. This information landscape is further complicated by the clonal nature of corals which is leveraged in many coral propagation and restoration programs wherein each *de novo* collection event can result in a clonal lineage distributed widely among programs, habitats, and geography.

For each of these coral restoration stakeholder groups, the fundamental unit being tracked is a unique instance of an observed coral – a colony of a certain genotype – identified or collected at a specific place and time. While each group is generating and tracking important information about the biology and restoration utility of specific strains of coral, this information is often isolated in idiosyncratic data storage systems that are agency- or project-specific. For example, the few restoration groups based in the Florida Keys each maintain their own data structures detailing collection, nursery, outplant, and performance for any given genotype. While coral fragment swaps between groups do occur, these data are rarely combined into one central database. Rather, shared data are duplicated across groups and remains siloed.

Presently, access to information across all systems is not possible due to the lack of standard data fields, structure, and storage capacity, making it difficult to leverage the collective knowledge across groups for informed adaptive management decisions. **Figure 2** provides a partial list of data structures generated within just a small geographic range of groups working toward management, research, and restoration goals for corals along the Florida Reef Tract. In addition to the physical isolation of datasets, issues of data integrity, disparate naming conventions, and even knowledge of what information is available confound the problem. Thus, access and adjudication issues slow the spread of knowledge even in those cases where there is willingness to invest in cross-platform integration.

Adaptive management for coral reef restoration initiatives will depend on the ability to access the broadest collection of data possible, as efficiently and quickly as possible. To that end, information associated with specific coral restoration activities must be accessible across organizations managing, researching, and working with those strains. As the first step in addressing this problem, herein we present the Coral Sample Registry, a convenient, web-accessible centralized system whereby coral fragments used for management, research, or restoration can be registered at the time of collection and issued a unique identifier, the Accession Number. The accession number provides a common field which can be used to standardize the way various groups communicate about the same information and

Data Category	Data Type (pertaining to ONE sample)	Restoration Practitioners	Academic Researchers	Resource Management Agencies
SAMPLE COLLECTION	*Collection date*	X	X	X
	Collection location	X	X	X
	Collection personnel	X	X	
	Collection reef name	X *	X *	X *
	Collection reef habitat		X	
	Collection permit	X *	X *	X *
	Local sample name (often, putative genotype)	X * ^	X *	X *
WILD COLONY	*Parent colony status (alive, dead)*	X ^	X ^	X ^
	Parent colony disease susceptibility		X *	X *
	Parent colony bleaching resistance		X *	
	Parent colony growth rate		X *	
	Parent colony sequencing completed (yes, no)		X *	
	Distance from other sampled colonies	X * ^	X * ^	X * ^
	Total number of collections from parent colony			X * ^
NURSERIES	*Nursery structure type*	X *		
	Nursery location	X *		X
	Quantity of fragments	X * ^		
	Holding nursery(ies)	X * ^		
	Nursery colony status (alive, dead)	X * ^		
	Nursery colony performance		X *	
	Restoration purpose (i.e. gene bank or propagation)	X *		
	Transfer quantity and size to outside group(s)	X * ^	X * ^	X * ^
OMICS	*Sequencing completed (yes, no)*	X *	X *	X *
	Coral genotype	X*	X*	X*
	Gene expression information		X*	
RESTORATION OUTPLANTING	*Outplant sites*	X * ^		X * ^
	Outplant quantities	X *		X *
	Outplant dates	X * ^		X * ^
	Outplant technique(s)	X * ^		X * ^
	Outplant size(s)	X * ^		X * ^
	Outplant status (alive, dead)	X * ^		X * ^
	Outplant performance	X * ^		X * ^
	Outplant sampling events	X * ^		X * ^
RESEARCH TRIALS	*Research restoration sites*		X *	X
	Research restoration treatments		X *	X
	Research colony performance		X *	X
	Research colony disease susceptibility		X *	X
	Resarch colony bleaching resistance		X *	
	Research colony growth rate		X *	
	Research colony fusion rate		X *	
	Laboratory colony performance		X *	
SPAWNING	*Nursery colony spawning*	X * ^	X * ^	
	Nursery colony gametes collected	X * ^	X * ^	X * ^
	Outplant colony spawning	X * ^		X * ^
	Outplant colony gametes collected	X * ^	X * ^	X * ^
	Colony parent gametes	X	X	X
	Gamete cryopreservation	X *	X *	X

KEY:

X : Data used by Group

***** : Indicates data types (eg. designation, defintion, naming convention etc.) that may change between groups for the same sample

^ : Indicates data types where one group may have multiple observations over time for a single sample.

FIGURE 1 | Data types collected and used in coral restoration efforts across Restoration Practitioners, Academic Researchers, and Resource Management Agencies. Each of these broad categories may represent multiple groups working concurrently. For example, in Florida, resource management agencies could include NOAA Restoration Center, Florida Keys National Marine Sanctuary, Florida Fish and Wildlife Conservation Commission, Army Corps of Engineers, and the Department of Environmental Protection. *Indicates data types that may change between groups for the same sample. ^Indicates data types where one group may have multiple observations over time for a single sample.

Practitioner Collection Data Structures	*CRF Collections* *FWC Collections* *Mote Collections* *NPS Collections* *NSU Collections* *Reef Renewal Collections* *Rescue Corals Collections* *TNC Collections* *UM Collections*
Existing Data Structures	*Acropora Outplant Database - NOAA SERO* *Annual Permit Reporting data structures - FKNMS* *Acropora Presence or Absence Locations - FWC* *Atlantic and Gulf Rapid Reef Assessment - AGRRA* *Caribbean Coral Spawning Monitoring Database - UNINMAR, ICML-UNAM* *Coral Portal - Gulf of Mexico Fishery Management Council* *Coral Reef Evaluation and Monitorign Program - FWC* *Coral Rescue - Coral Monitoring Dashboard - FWC* *Dendrogyra cylindrus wild colony surveys - Cindy Lewis* *Disurbance Response Monitoring Database - Florida Reef Resilience Program - FWC* *Integration of Resource Management Applications - NPS* *National Coral Reef Monitoring Program - NOAA* *NOAA Acropora palmata Population Management Database* *NOAA AOML Coral Program physiological database* *NCCOS Spatial Modeling of threatened Caribbean corals - NCCOS* *Outplant & Monitoring Data stuctures - Practitioners (CRF, FWC, Mote, NPS, NSU, Reef Renewal, TNC, UM)* *Southeast Fishery Independent Survey - SEFSC* *STAGdb - Penn State*

FIGURE 2 | A subset of coral data structures related to Florida coral restoration efforts. Practitioner Collection Data Structures represent those which will be used to populate the Coral Sample Registry, each sample being assigned an accession number. Existing Data Structures are representative examples of how coral data is used; this representative list is specific to Florida and the Caribbean and not intended to be exhaustive of what may exist.

is associated with the sample thereafter across any and all data structures. The system is designed to be simple to use, independent of coral species or geographic location, and provide multiple means for entering and accessing information. The Coral Sample Registry is not intended to directly link the different data repositories, but to provide a standardized key corresponding to unique coral samples that can then be used to unlock the information across different data repositories.

MATERIALS AND METHODS

The concept for the Coral Sample Registry (CSR) was developed at an initial meeting with key stakeholders at the Reef Futures 2018 conference held in Key Largo, Florida. Representatives included restoration-practitioner groups, academic researchers, and United States resource management agencies at the federal and state levels, participating to discuss how to more efficiently access the various data streams being generated in order to better inform adaptive management of the restoration efforts occurring across the Florida Reef Tract. Four principles were agreed upon to guide this work. First, any solution should be accessible regardless of coral species or geographic location. Second, to the extent possible, best-practices for data-repository construction and management should be employed. Third, any solution should be easy to use and not impair the ongoing data management activities of existing stakeholders. Fourth, given the landscape of complex and diverse data structures already in existence, a more generalized solution was preferable to a specific one.

Based on the principles outlined, we determined the simplest and most effective solution was a system whereby individual coral samples could be assigned a unique identifier (hereafter an accession number) that could be incorporated into existing data structures. In this manner, the accession number would serve as a hashtag allowing information in different data repositories to ultimately be integrated. Moreover, it would require minimal modification to existing data structures and no need to transfer or duplicate current data to a new platform. The CSR was designed with this narrow scope in mind: to be a registry of coral samples used in various restoration activities and to assign each sample a unique accession number. It is not intended to be an aggregator of all information or even to directly link existing data structures; it is intended to provide a common key that can be integrated into existing data structures to allow cross-linking in the future based on needs.

With this framework in mind, key aspects of the CSR are described below. Additional details can be found in the User's Guide document associated with the website.

Scope

The Coral Sample Registry has been developed to accommodate corals of any species in any geographic region. Although the project began with a focus on South Florida restoration, the final product is suitable for use globally.

Access

Hosted at: https://www.crfcoralregistry.com.

Defining a Unique Sample for Assignment of Accession Numbers

A unique sample (i.e., base unit) is defined as the unique combination of six fields (defined later in the text): Sample

Type, Local Sample Name, Collection Date, Genus, Species, and Organization. This represents the base unit to which an accession number is assigned.

The base unit defined in this manner differentiates collection of samples from the same wild colony at two different time points (or agencies), each sample receiving a unique accession number. This avoids the assumption that local samples, which might be subjected to different post-collection analysis or subsampling, be artificially conflated. The system provides sufficient flexibility to allow wild collections or sexual crosses to be registered and receive accession numbers.

It is important to realize the base unit is a collection event and may not equate to a unique genotype. We believe that this is an important and powerful feature of the CSR. It allows a sample (of a putative genotype) to be tracked prior to any investment in genotyping. The dominant practice outside of research is to archive collected samples prior to genotyping, and many small restoration and management practitioners around the world may never have their samples genotyped (pers obs.). If samples are subsequently found to be the same genotype based on a common methodology, then this information can be tracked and adjudicated outside of the CSR, such as in the data structure associated with the genotyping method. Following best practice, we have intentionally avoided allowing the post-collection association of a "genotype" to registered samples in order to avoid conflicting sequencing methods or altering original entries. We believe deconfliction of potential synonymous genotypes is best done outside of the registry as these classifications may change as techniques and methodologies evolve.

Infrastructure

Amazon Web Services (AWS) is used for hosting the Coral Sample Registry website and database. AWS, which is a leader in cloud computing, provides security, high availability, and reliability for the Coral Sample Registry 24/7 across the globe.

Defined Users

Users of the CSR are classified as Registered Users. Registered Users can enter new sample information and access the full data repository. Registered users are required to apply for access using an email domain corresponding to their parent institution (e.g., @noaa.gov or @coralrestoration.org).

Data Entry and Data Access

Registered Users have two options when inputting data. Samples can be entered: (a) individually, entering each field through the web interface; or (b) via a bulk upload option using a pre-existing spreadsheet, for which a template is available. The bulk upload option recognizes errors and exports an error file for modification and re-upload, flags and prevents duplicate uploads, and offers an immediate export of newly added accession numbers for incorporation into the originator's databases.

Editing of Previously Entered Information

Entered data can be subsequently edited, if needed, only by the Registered User who made the entry. All changes are captured in the metadata. Entries cannot be deleted; hence accession numbers will never be re-assigned to a new sample.

Data Access and Viewing

Once data are uploaded, they are visible to all Registered Users on the CSR's browser tab. Data filtering and downloads are also made possible using the CSR's browser tab, where even the entire contents of the CSR can be downloaded. Should additional data about a specific entry be of interest, user contact information is made available.

Data can be searched using any field or any combination of fields. In addition, there are a limited number of pre-existing summary reports available for convenience, located under the Reports tab.

Data Integrity, Back-Up and Redundancy

The Coral Sample Registry data is stored in an isolated PostgreSQL relational database in AWS. Back-ups of the database are taken on a daily basis for disaster recovery. The data in the registry is replicated across multiple "zones" within the AWS network. This helps provide an additional layer of data redundancy in the event of a failure.

Data Fields

The following summarizes the data fields within the CSR architecture; additional details can be found in the Coral Sample Registry User's Guide, accessible online.

Accession Number

Generated field. An accession number is a randomly generated 36-digit alphanumeric string keyed using a randomized algorithm based on the server time. It is associated with a coral sampling event as described above (a unique combination of Sample Type, Local Sample Name, Collection Date, Genus, Species, and Organization fields).

Sample Type

Required field; constrained by picklist. This field consists of a limited list: the field can either state "Wild Colony" or "Sexual Recruit." A Wild Colony is defined as either a detached fragment of opportunity or a fragment taken from a wild colony. A Sexual Recruit is defined as a coral created by assisted sexual reproduction during which gametes were harvested, fertilized, and subsequently settled in a lab or nursery setting.

Latitude

Required field; limited text. Latitude corresponds to the location where the sample was obtained. Entries can be made in any of three formats (Decimal Degrees; Degrees and Decimal Minutes; or Degrees, Minutes, Seconds), though all are converted and standardized to Decimal Degrees upon successful upload. For Sexual Recruits, this refers to the location of larval settlement (i.e., nursery or lab).

Longitude

Required field; handled as per Latitude.

Country
Required field; picklist. Specifies the sovereign nation that the sample originated from. For Sexual Recruits, this field refers to the country in which the recruit was settled, thus the permitting sovereignty governing the sample's handling. This is a more consistent designation than country of larval origin since, with increasing application of cryopreservation and assisted gene flow in coral breeding, the time of collection and geographic origin of coral larvae will become increasingly complex (i.e., egg and sperm collections may come from different countries in different years).

Region
Optional field; free form text. Specification of the local region of the source sample.

Subregion
Optional field; free form text. Specification of the local subregion of the source sample.

Reef Name
Optional field; free form text. Specification of the local reef name of the source sample.

Site Name
Optional field; free form text. Specification of the local site name of the source sample, often a specific area of a local reef.

Genus
Required field; corrected free text. Entries are compared against a standardized list of coral genera and species (World Register of Marine Species) for correct spelling. Only recognized genus-species combinations are possible: mis-matched entries are flagged for review.

Species
Required field; handled as per Genus.

Local Sample Name
Required field; free form text. This refers to the name or ID assigned to a sample by the collecting organization. This likely represents the putative genotype.

Collection Date
Required field; constrained format. For a Wild Colony, this refers to the date of collection from the wild. For a Sexual Recruit, this refers to the date of settlement.

Notes
Optional field; free form text. This field is used to incorporate pertinent information about a collection event. This includes, but is not limited to, information about the possible parents of a sexual recruit, information on the status of the parent wild colony from which a fragment was taken, or additional collection information that does not fit into the above fields.

Submitter
Generated field. Provides the name of the Registered User who made the entry, referenced from their account information.

Organization
Generated field. Provides the name of the Organization of the Registered User who made the entry, referenced from their account information.

Contact Information
Generated field. Provides the email address of the Registered User who made the entry, referenced from their account information.

DISCUSSION

Protecting coral reefs and enhancing coral populations in the face of further anthropogenic change requires deeper insights into the biology of coral species and their ecological communities. As data are generated across various fields and multiple researchers, we face the challenge of integrating this information into actionable management strategies, hopefully to outpace the loss of coral cover. Reducing the amount of time it takes for collected data to become actionable, by accessing and integrating the broadest collection of information possible, will be necessary for success. The Coral Sample Registry is designed as an essential first step toward this goal, providing a means to cross-reference – and thereby access – disparate information types related to coral samples.

The creation of the CSR accomplishes four major things for the field of coral conservation. First, it establishes a single, permanent record of coral samples. Entries will not be deleted from the registry, but new ones can be added at any time, making the CSR an up-to-date database of collections made across groups, species, and regions. Currently, no data structures exist that meet this need. While powerful individual research tools, databases for genotype or restoration-nursery collection information are successful at capturing high resolution information for large numbers of corals, but do not incorporate all collected samples, only subsets that qualify (e.g., have been genetically analyzed). Second, it standardizes the minimum set of information related to coral samples, regardless of group, species or region. This information directly addresses a need outlined by the Coral Restoration Consortium 2020–2025 priorities to better define terms associated with coral restoration for improved management (Coral Restoration Consortium, 2021; Vardi et al. in review).

Third, it offers a single point of reference for where coral samples have been accessioned, thus allowing spatial gaps in collections or potential redundancies to be easily identified while drawing attention to potential collection overlaps between groups. Presently, knowledge sharing about sampling events requires intensive effort from multiple groups to maintain several databases, each updating at different times. Rather than relying only on resource management agencies to provide population-level metrics about coral fragments in use by all groups, the CSR offers an up-to-date structure easily accessible by all parties. Finally, as an open-access repository, it allows for all participating groups and resource management authorities to share and access information collaboratively to tackle broad problems as a unified community, rather than fractured segments.

The CSR provides a convenient method to accurately communicate among different data structures but does not guarantee mutual access or cross-platform integration of these sources. We recognize that while the CSR offers the potential for greater information integration and access, the challenge will be with its broad adoption. Key to the success of the CSR is the widespread registration of coral samples within the registry from all groups, the pairing of an accession number with how coral samples are used over time, and the inclusion of an accession number field in data structures currently in use or in development to track a fragment's origin. Adoption at each of these levels can be daunting. However, we remain optimistic given the growing desire to leverage coral-level information across all parties for both research and management.

For researchers, the ability to directly link the source of their collection material with the various attributes of individual strains is critical in elucidating genome by environment responses associated with various stress challenges. Interventions such as assisted migration, assisted gene flow, and selective breeding activities are becoming increasingly viable interventions (National Academies of Sciences Engineering and Medicine, 2019), necessitating a link between collection information, genomic, and phenotypic information of individual samples. Therefore, we actively encourage research groups to incorporate a field corresponding with the CSR accession number into their current data repositories. Through incorporation of the common accession number information, academic researchers would be able to access all collection information for samples under current study or previously studied, allowing for comparison across multiple research project-specific datasets that examine many different aspects of a particular coral strain (e.g., heat stress tolerance, disease susceptibility, growth rates). Several groups have already incorporated a blank field to be populated by the generated accession numbers such that their databases can be immediately cross-referenced using the common key provided by the CSR. Examples of these data structures are the NOAA *Acropora palmata* Population Management Database, Pennsylvania State University Acroporid Genotype Database, the Caribbean Coral Spawning Monitoring Database, and the NOAA AOML Coral Program physiological database in development.

For restoration practitioners, the CSR provides a free, easy to use resource to monitor their collection activities and inventory collected samples. By directly tying a collection event to an initial coral sample, a clear link is established between the collection event and the subsequent lineage of that sample through asexual reproduction, currently the dominant form of propagation for many coral restoration practitioners (Boström-Einarsson et al., 2020; Hein et al., 2021). As we learn more about the phenotypic plasticity of various coral species, it is becoming clear that the variance associated with a particular genotype in different environments is complex. Therefore, understanding the survivorship and performance of the same genotype collected from different locations or times may be an important co-variance factor. Tracking these lineages for testing and observation is a growing priority, facilitated by the CSR. Swapping of coral strains among research groups and/or practitioners, as is common in areas with large restoration programs like the Florida Reef Tract, is an increasingly important means of diversifying populations. However, this beneficial practice can become problematic if there aren't sufficient controls to ensure transparent transfer of collection information, as is guaranteed via a registered accession number. Restoration practitioners would incorporate the accession number into all existing data structures, allowing for a long-term data analysis of strains outplanted across multiple years in different locations and quantities. We encourage all restoration practitioners to register all current and future samples in the CSR, especially during coral swaps between groups.

For resource managers, including governmental permitting agencies, the CSR provides an essential tool to ensure efficient coordination of restoration efforts while protecting natural populations. A resource such as the CSR provides readily available summary information on a sample's origin and therefore an estimate of the relative diversity of coral stocks across organizations without having to invest in development of new systems. The CSR is designed to be integrated into existing management systems, either through manual uploads or a direct application programming interface. The CSR removes the burden of sharing collection and stock information from the management agency by placing it in a publicly accessible forum, which also facilitates better coordination. Resource management agencies will be able to mine all registered samples in an area to gain an understanding of overlap between groups, across species, or perhaps identify areas that should be scouted for unknown wild colonies. The CSR provides a tool for managing corals as a population rather than group-owned stocks. As new territories and nations expand their coral restoration efforts, we encourage the inclusion of CSR registration as part of their permitting pipeline.

A potential benefit of the CSR that spans all stakeholder groups concerns recordkeeping and analysis in the case of natural resource damage. Legal remedies for such damage caused by an anthropogenic event require strict chain-of-custody for samples that can be greatly facilitated by the CSR, while simultaneously providing a one-stop record of existing pre-disaster samples that might be accessed for reference. As an example, the 2010 Deepwater Horizon oil spill prompted emergency coral sampling in advance of an anticipated arrival of oil contamination via the Gulf Stream. The *de novo* invention of a chain-of-custody system for these samples was a significant component of this effort. With a functional CSR in place, this recordkeeping effort (and to some extent the sampling effort as well) may have been much reduced.

Finally, we encourage funders and publishers to encourage coral-related submissions to register their samples with the CSR and track accession numbers, as is common practice for permits and samples. In this way, information can be transparent and publicly accessible, enabling investments and outcomes to have the broadest possible impact.

The needs for standardized terms and metrics across coral restoration as well as the data management structures to collect, store, and share key data are well defined by coral restoration

management agencies (Coral Restoration Consortium, 2021; Florida Fish and Wildlife Conservation Commission, 2021). The Coral Sample Registry helps to fill these needs by correlating information related to coral samples across multiple sources. By itself it can serve as an invaluable tool to further collaboration, document and track the origin of restoration materials, provide insight into the sampling of wild populations, and facilitate knowledge sharing among groups. Collectively, this helps close an important knowledge gap, increasing confidence that a complete picture of sampling efforts is available, lowering the risk of missed information. But the CSR's efficacy will depend on its adoption as a repository for sample collections, and the subsequent association of sample accession numbers in derivative efforts. Toward the greater good of considering collected samples as part of a large meta-population, we encourage restoration practitioners, researchers, and management agencies to adopt the CSR accession number standard, institutionalizing its inclusion where possible. Alone, the CSR represents the potential for greater insights; it will be up to the broader community to use the accession number as a key to unlock information across data repositories.

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

AmM managed the project's development and produced the original draft of the manuscript, with significant contributions from BB, RD, LM, MM, JM, AlM, and RW. All authors contributed equally to the design of the project.

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METHODS
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Assisted Coral Reproduction in the Dominican Republic: A Successful Story to Replicate in the Caribbean

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Coral assisted fertilization, larval rearing and recruit propagation success in significant ecological scales, largely depend on scaling up and replicating these efforts in as many regions as possible. The Dominican Foundation for Marine Studies (FUNDEMAR) has become a pioneer of these efforts in the Dominican Republic, being the first institution to successfully implement coral sexual reproduction techniques in the country and establishing the first mobile larvae culturing facility. Here we share our perspective on three main components behind the success of FUNDEMAR's program: (1) a self-sustainable program in alliance with local and international organizations, (2) the design and construction of the first Coral Assisted Reproduction Laboratory in the country, and a (3) clearly defined scalable structure for outcome performance. Two years after program implementation, FUNDEMAR has successfully produced an annual regional coral spawning prediction calendar, cultured seven coral species, and seeded over 4,500 substrates with more than 268,200 sexual coral recruits in approximately 1,880 m^2 reef areas. Here, we provide a detailed description of a fully functional assisted coral reproduction program, including the lessons learned during its implementation as well as a series of specific solutions. We hope this work will help and inspire other countries and small institutions to replicate FUNDEMAR's coral assisted reproduction program components and contribute to the expansion of sexual coral restoration efforts in the Caribbean.

Keywords: coral restoration, coral reefs, sexual propagation, coral rearing, science-based solutions, Dominican Republic

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INTRODUCTION

During the past few years, coral restoration efforts have increased globally (Boström-Einarsson et al., 2020) and across many Caribbean and Latin-American countries (Bayraktarov et al., 2020). Coral restoration has been proposed as a solution to decrease and/or ameliorate the impacts of local and global stressors as a useful tool to preserve reefs, recover populations of reef building corals or both (Calle-Triviño et al., 2018; Bayraktarov et al., 2020; Boström-Einarsson et al., 2020; Goergen et al., 2020; Shaver et al., 2020).

Coral restoration can be done through asexual and sexual propagation and other types of interventions to enhance substrate suitability for outplanted or gardened corals (Boström-Einarsson et al., 2020). While asexual fragmentation can rapidly increase coral tissue coverage, it does not directly increase genetic diversity in coral populations, making threatened populations potentially vulnerable to diseases and environmental stress (Barton et al., 2015). On the other hand, sexual propagation may increase genetic diversity and therefore resilience, however, low recruit survival after seeding to a reef is still a challenge for restoration practitioners (Baums et al., 2019). Regardless of the method or the approach used, restoration programs still face challenges often associated with the problem of scaling up efforts in space and time while preserving genetic diversity of wild coral populations (Boström-Einarsson et al., 2020).

Recent advances on larval enhancement techniques wouldn't have been possible without the cumulative general knowledge of coral biology and ecology (Guest, 2010; Guest et al., 2014). Reproduction in corals consists of a sequence of events which include gametogenesis, spawning (for spawning species), fertilization, embryogenesis, planulation, dispersal, settlement, and recruitment (e.g., Harrison and Wallace, 1990; Baird et al., 2009; Harrison, 2011). These events have been described from different perspectives, including histological (e.g., Duerden, 1902; Fadlallah, 1983; Szmant, 1986; Richmond and Hunter, 1990; Soong, 1991; Steiner, 1998; Morales, 2006; Ritson-Williams et al., 2009; Weil and Vargas, 2010; Harrison, 2011; Soto and Weil, 2016), observational (e.g., Vermeij et al., 2003; Levitan et al., 2004; Van Woesik et al., 2006; Vize, 2006; Bastidas et al., 2012; Chamberland et al., 2016; Keith et al., 2016; Fogarty and Marhaver, 2019), and experimental (e.g., Morse et al., 1988; Webster et al., 2004; Kuffner et al., 2006, 2007; Nugues and Szmant, 2006; Vermeij et al., 2009; Ritson-Williams et al., 2010; Marhaver et al., 2015; Sharp et al., 2015) studies.

Early attempts to incorporate the concept of larval propagation for restoration purposes started two decades ago (Rinkevich, 1995; Petersen and Tollrian, 2001). In the Caribbean, first successful attempts to rear larvae in the laboratory were conducted by Szmant and Miller (2006) in the Florida Keys and by Randall and Szmant (2009) in Puerto Rico. Currently, Coralium lab at the National Autonomous University of Mexico, SECORE International and the Caribbean Research and Management of Biodiversity (CARMABI) are three leading institutions in the Caribbean on this subject. These institutions have played an important role in expanding larval propagation across geographies, improving capacity building, and developing cutting edge technology and protocols applied for coral restoration (e.g., Marhaver et al., 2015; Chamberland et al., 2016, 2017; Banaszak pers. comm.).

The Dominican Foundation for Marine Studies (FUNDEMAR) has been able to locally adapt these techniques integrating an assisted sexual coral reproduction program, the first one in the Dominican Republic (Calle-Triviño et al., 2018). Here, we provide our perspective explaining the success of FUNDEMAR's program: (1) a self-sustainable program in alliance with local and international organizations, (2) the design and construction of the first Coral Assisted Reproduction Laboratory in the country, and (3) a clearly defined scalable structure for outcome performance.

KEY COMPONENTS BEHIND THE SUCCESS OF FUNDEMAR's PROGRAM

FUNDEMAR's Self-Sustainable Coral Restoration Program

FUNDEMAR was founded in 1991 with the mission to preserve marine ecosystems in the Dominican Republic. It is based in Bayahibe, a small town of fishermen which is also a hotspot for tourism activities in the country (**Supplementary Figure 1**). The early strategic alliance between FUNDEMAR and the tourism private sector was a key step for achieving a sustainable program. The first couple of alliances in our coral conservation program motivated and prompted other local stakeholders (hotels, resorts, dive centers, and the community) to get involved. In time, the stakeholders themselves became emotionally engaged with FUNDEMAR to preserve coral reefs resilience for its intrinsic value and for its services to local people.

This structure was consolidated not only with the support of the private sector (economically and in kind) but also by FUNDEMAR's initiative to create permanent income mechanisms (such as hosting educational programs with international students amongst other fundraising strategies). As a result, since 2012 to date, FUNDEMAR has gradually scaled up the program as it was becoming more sustainable. The coral program relies on four pillars: (1) research and monitoring, (2) asexual (Calle-Triviño et al., 2020) and sexual restoration (Calle-Triviño et al., 2018; **Supplementary Figure 2**), (3) management, and (4) community integration and awareness. FUNDEMAR's coral restoration program (**Supplementary Figures 1,2**) is only one of a series of interconnected marine conservation programs that complement each other for preserving coastal ecosystems within the Southeastern Reefs Marine Sanctuary.

In 2017, FUNDEMAR joined SECORE's capacity building program as an implementation partner. Since, SECORE has provided restoration technology and training on larval prorogation to FUNDEMAR staff. This alliance and the partnership with The Nature Conservancy's coral strategy, placed the Dominican Republic as a key location to scale up assisted fertilization and larval propagation efforts in the Caribbean, integrating SECORE's unique Coral Rearing *In Situ* Basins (CRIBs, SECORE International, 2020; **Supplementary Figures 2G,H**) and substrate designs (Chamberland et al., 2017; **Supplementary Figure 3**), amongst other technologies.

Design and Construction of the First Coral Assisted Reproduction Laboratory in the Dominican Republic

In 2019, FUNDEMAR established a mobile *ex situ* coral assisted reproduction laboratory by adapting a storage container (12 m × 2.44 m × 2.6 m L, W, H) into a fully functioning laboratory for rearing corals (**Supplementary Figures 2E,F**). Inspired by the experience and knowledge gathered from

trainings in Curaçao by SECORE and in Mexico by CORALIUM and SECORE, various FUNDEMAR partners helped design and build this facility for its intended goal. The main adaptations involved lining the walls with a thermal insulator, running electrical lines, plumbing for fresh and sea water flow and installing fans and air conditioning for temperature regulation. The wet lab itself consists of three major components: (1) a water catchment system, (2) a filtration system, and (3) an aquarium system. An in-depth description of the laboratory including design and system functioning is provided in the **Supplementary Material** (**Supplementary Figure 4**).

Furthermore, the CRIB technology allowed us to escalate the production of coral settlers. CRIBs consist of three main parts: (1) a floatable hydrodynamic ring that maintains the structure and is anchored to the bottom, (2) a canopy located above the floatable ring that protects the embryos from ultraviolet radiation as well as fresh water in case of rain, and (3) an underwater vertical enclosure with removable mesh windows (100 and 200 μm) to allow continuous water exchange (**Supplementary Figures 2G,H**).

Clearly Defined Scalable Structure for Outcome Performance

FUNDEMAR's program structure has been capable of scaling up gradually based on clear annual goals defined in the institution's restoration plan, which is annually evaluated. During the first 2 years of program implementation (2019–2020), efforts were mainly focused on the standardization of coral sexual culturing techniques as well as building capacity for FUNDEMAR staff and other coral restoration practitioners. Yet, we were able to scale up and enhance outcomes from 1 year to the next (presented below). We also focused on gathering enough spawning documentations to create our own coral spawning prediction calendar. The first calendar was available in 2019 and it is updated every year to increase reliability in gamete collection locally.

In Southeastern Dominican Republic, most massive coral spawning events (>25% of colonies spawning) occurred in May and June for *Diploria labyrinthiformis*, August for *Acropora palmata*, *A. cervicornis* and *Dendrogyra cylindrus,* and September for *Colpophyllia natans, Orbicella annularis* and *O. faveolata. D. labyrinthiformis* had the earliest spawning events while the acroporids and *Orbicella* spp. spawned the latest. For most species, a few colonies spawned a little the day before their massive spawning event. Spawning patterns were less stable through time and had longer windows for acroporids compared to other species (**Supplementary Table 1**).

From 2019 to 2020, gametes were collected, eggs fertilized, embryos cultivated, larvae settled, and recruits seeded from five different coral species*: D. labyrinthiformis*, *D. cylindrus* (Villalpando et al., 2021), *A. palmata*, *A. cervicornis*, and *C. natans* (**Figure 1**, **Table 1**, and **Supplementary Table 2**). In 2020, gametes from *O. faveolata* and *O. annularis* were also collected and successfully fertilized, however, the larvae culture died during the swimming stage in both *in situ* and *ex situ* cultures for unknown reasons.

The implementation of both *in situ* and *ex situ* culturing systems allowed for a high production of substrates with coral sexual recruits or seeding units (SUs: *sensu* Guendulain-Garcia et al., 2016; Chamberland et al., 2017). In 2019, 1,927 SUs with 28,900 coral settlers were seeded in 850 m^2 of reef. In comparison, in 2020, 2,615 SUs with 239,300 settlers were seeded in 1,030 m^2 of reef. In total for both years, over 4,500 SUs with approximately 268,200 sexual coral recruits were seeded in reef areas totaling around 1,880 m^2 (**Supplementary Table 2**). These results were achieved with systems below their maximum capacity (i.e., *ex situ* system 1,000 substrates and CRIB ∼2,000).

In the middle to long term, we expect to produce a database to test specific hypotheses regarding which species and substrate type show higher recruit survival and which are more cost-effective to reproduce.

DISCUSSION

In this paper we provide our perspective about a series of guidelines that are needed to implement a sexual propagation restoration program through assisted coral reproduction. We find these 3 points to be essential to create a robust and scalable program: (1) alliances with private and local stakeholders, (2) financial stability, and (3) adoption of novel technology supported by pertinent training to implement them. In our view, the balance between these components allowed FUNDEMAR to build the laboratory and produce results comparable to others in the Caribbean (Chamberland et al., 2016, 2017). Local engagement and key alliances between different stakeholders and the scientific community has been shown to be a strong pillar that supports conservation actions aimed to preserve coastal marine ecosystems (White and Vogt, 2000; Lundquist and Granek, 2005; Reyes-García et al., 2019).

In our perspective, the most valuable lessons learned during the implementation of our sexual propagation program are logistical, technical, and structural. These lessons are often interconnected and must be taken into account holistically as the restoration program is implemented.

From the logistic point of view, getting permits on time is a key step. FUNDEMAR has been able to obtain environmental permits to implement these actions, based on our solid reputation of contributing and supporting the national strategy for coral conservation in the Dominican Republic. Environmental hazards and catastrophes such as hurricanes may impose logistical problems, and in some cases, delay restoration efforts. The best way to cope with the problem is to create an action plan that can be carried out in various seasons and includes more than one species for collection and fertilization as well as diverse cultivation methods to reduce vulnerability to climatic, environmental or unforeseen events, always having a backup plan.

The combination of differing culturing facilities and settlement substrates designs was crucial to increase the production and upscaling the restoration effort. This allowed us to seed a substantial amount of SUs in larger reef areas. The use of the CRIBs represents a feasible strategy for mass production of SUs, nevertheless it is important to consider the local environmental conditions with this method. This type of system works well in relatively calm waters and having an emergency plan for adverse weather conditions is fundamental; in 2019 and 2020, we removed the CRIBs as a hurricane prevention

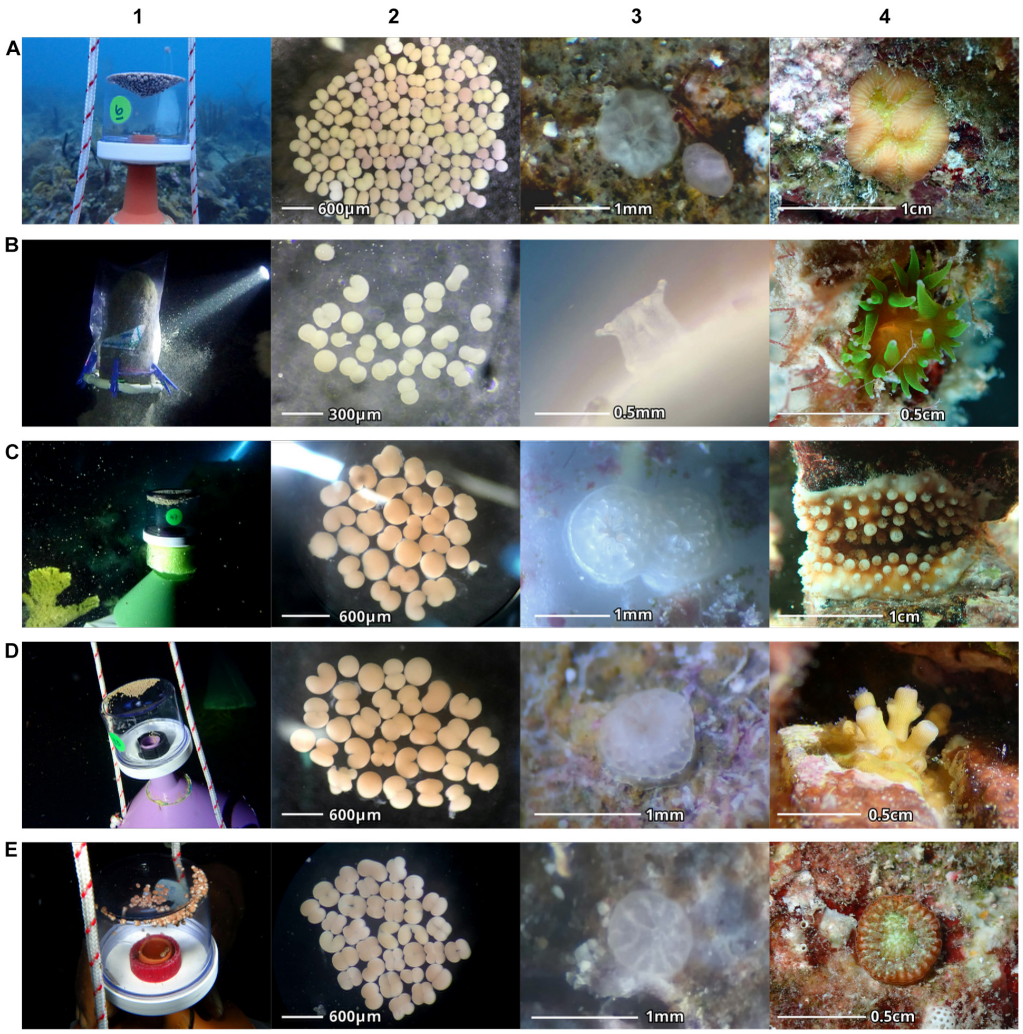

FIGURE 1 | Coral assisted sexual reproduction phases **(1)** Gamete collection **(2)** Fertilized embryos **(3)** Primary polyps and **(4)** Coral recruits after 6 to 12 months of seeding for the five reared coral species: **(A)** *Diploria labyrinthiformis* **(B)** *Dendrogyra cylindrus* **(C)** *Acropora palmata* **(D)** *Acropora cervicornis* **(E)** *Colpophyllia natans*. (Photo A3: Paul Selvaggio/PghZoo/SECORE).

measure, and sheltered the substrates in the laboratory as well as in underwater structures used for preconditioning substrates.

As for the technical lessons, initial time investment in monitoring local spawning events is essential to accurately forecast them, reducing costs in the long term by making time in the field more efficient. This in turn leads to better working plans and therefore a reduction of potential human errors that may compromise the success of gamete collection, fertilization, and culture in the laboratory. Also, the adoption of an experimental design framework and the standardization of protocols to assist coral reproduction is highly recommended. In terms of experimental design, having a clear formulation of hypothesis for the restoration experiment based on the comparison of variables measured in experimental, reference and control plots effectively estimates restoration success (Chapman, 1998; Croquer et al., 2019). Furthermore, sampling efforts should be established *a priori* based on power analysis.

It is critical to acknowledge that coral restoration by itself is not the solution for preventing local coral reef decline. Instead, coral propagation must be aligned with specific management strategies aimed at reducing local threats such as overfishing, water pollution, unsustainable coastal development and direct physical damage to the reef such as anchoring (Abelson et al., 2020). One of the advantages of FUNDEMAR's coral restoration approach is the holistic strategy implemented to protect Southeastern Dominican reefs. Though local actions are essential, a coordinated international approach is necessary to reduce global threats to coral reefs such as mass coral bleaching and ocean acidification caused by climate change (Abelson et al., 2020).

To conclude, FUNDEMAR's coral assisted sexual propagation program has become a robust program due to key alliances entailing the private sector, NGOs and governmental agencies, the engagement of the local community, the creation of

TABLE 1 | Assisted reproduction output from 2019 and 2020.

Species	Spawning year	Spawning date	DAFM	TAS	No. colonies collected	Min. to 80–90% Fert.	MFR (%)	Approx. No. Embryos (Thousand)	Culture method	Days in culture
Dlab	2019	May 28	10	1:40**	5	–	–	–	*Ex situ*	14
		May 29	11	1:10**	18	80	100	1,500	*In situ*	13
								–	*Ex situ*	
	2020	May 17	10	1:05	4	80	96.2	498	*Ex situ*	18
		May 18	11	1:00	10	80	94.6	301	*Ex situ*	17
		Jun 16	11	1:15	11	80	96.5	457	*Ex situ*	13
Apal	2019	Aug 17	2	2:15	10	220	90	–	*In situ*	24
									Ex situ	
	2020	Aug 7	4	2:05	10	160	84.8	328	*In situ*	21
		Aug 8	5	2:20	9	180*	74.5	118	*Ex situ*	21
								477	*In situ*	20
Dcyl	2019	Aug 18	3	1:35	4	60	83.3	–	*Ex situ*	43*
	2020	Aug 6	3	1:45	3	80	85.9	–	*Ex situ*	25
Acer	2019	Aug 20	5	2:35	9	180*	67.8	–	*In situ*	28
									Ex situ	
	2020	Aug 7	4	2:35	9	160	88.5	57	*Ex situ*	21
Cnat	2019	Sep 20	7	1:00	8	140	90	–	*Ex situ*	25
	2020	Sep 8	6	0:35	5	80	97	410	*In situ*	23
Ofav	2020	Sep 7	5	2:20	8	180	87.1	516	*In situ*	–
								–	*Ex situ*	
Oann	2020	Sep 7	5	3:25	8	120	92.2	–	*Ex situ*	–

DAFM, Days After Full Moon; TAS, Time After Sunset (hour:minutes); and MFR, Maximum Fertilization Rate. In 2020, Orbicella annularis and O. faveolata developed into larvae but, inexplicably, all cultures died off before settlement. Species name abbreviated to first letter to denote genus and first three letters of species.
**In 2019 Dendrogyra cylindrus was ready to be seeded earlier but was kept in culture for a longer time to monitor progress. **Time Before Sunset.*

job opportunities and training to become coral restoration technicians, and the creation of sources of income that make the program self-sustainable. The program relies on clear missions and objectives and integrates local stakeholders. Finally, the key of a successful and replicable program is to adapt the existing technologies and customize it to the reality of each country, the conditions of the site where it will be implemented and the technical and economic capacity of the institution.

FUNDEMAR is expected to continue growing in the near future and will continue sharing experiences with other NGOs in the Dominican Republic and the Caribbean region. We hope our story will be useful for others to design their programs and to replicate efforts across the region to scale up coral reef restoration efforts.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RS-B conceived, designed and coordinated FUNDEMAR's coral reproduction program, designed and led the Coral Assisted Reproduction Laboratory, acquired funds and resources and performed field work, supervised the data collection and execution of all activities, and contributed equally to the manuscript. MV led data collection, processing and analysis, elaborated the spawning prediction calendars, performed the field work, created figures and tables, and contributed equally to the manuscript. SG-G designed, led, adapted, and improved the construction of the Coral Assisted Reproduction Laboratory, performed field work, trained the technicians for the data collection, supported the creation of figures and tables, and contributed equally to the manuscript. AC contributed to the manuscript conception and structure, performed field work, provided support for the data analysis process, and contributed equally to the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Site Selection for Coral Reef Restoration Using Airborne Imaging Spectroscopy

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Over the past decade, coral restoration efforts have increased as reefs continue to decline at unprecedented rates. Identifying suitable coral outplanting locations to maximize coral survival continues to be one of the biggest challenges for restoration practitioners. Here, we demonstrate methods of using derivatives from imaging spectroscopy from the Global Airborne Observatory (GAO) to identify suitable coral outplant sites and report on the survival rates of restored coral at those sites. Outplant sites for a community-based, citizen science outplant event in Bávaro, Dominican Republic, were identified using expert-defined criteria applied to a suitability model from data layers derived from airborne imagery. Photo quadrat analysis of the benthic community confirmed the accuracy of airborne remote sensing maps with live coral cover averaging 3.5–4% and mean algal cover (macro algae and turf) ranging from 28 to 32%. Coral outplant sites were selected at 3–7 m depth with maximized levels of habitat complexity (i.e., rugosity) and live coral cover and minimized levels of macroalgal cover, as predicted by the imaging spectrometer data. In November 2019, 1,722 *Acropora cervicornis* fragments (80–180 mm in length) were outplanted to these sites. Surveys conducted in January 2020 in four of these sites confirmed that 92% of outplants survived after 3 months. By October 2020 (11 months after outplanting), survivorship remained above 76%. These results demonstrate higher than average success rates for coral outplant survival for this species. An online tool was developed to enable replication and facilitate future selection of coral restoration sites. Our objective is to present a case study that uses GAO-derived map products within a suitability model framework to provide a quantitative and replicable method for selecting coral restoration sites with the goal of increasing outplant survival over time.

Keywords: reef restoration, Caribbean, remote sensing, coral survival, imaging spectroscopy, suitability modeling, coral outplanting

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INTRODUCTION

Coral reefs provide tremendous economic benefits to coastal communities around the world, including fisheries production, tourism revenue, and coastal protection. Although they occupy less than 1% of the world's ocean area, coral reefs are one of the most diverse ecosystems on the planet, providing essential habitat for one-quarter of all known marine species (Plaisance et al., 2011).

Despite decades of continued effort to protect and restore coral reefs, these ecosystems continue to decline under a growing array of local and global threats, such as overfishing, pollution, and climate change-driven temperature extremes (Burke et al., 2011; Wear, 2016). The rapid decline of reef-building corals has led to a concomitant loss of structural complexity and biodiversity (Alvarez-Filip et al., 2009), reducing the ability of coral reefs to deliver critical ecosystem services that contribute to the well-being and economic livelihoods of millions of people (Hughes et al., 2010, 2017, Hoegh-Guldberg et al., 2019). While this trend has been increasingly recorded across the globe, Caribbean reefs have been among the most severely impacted during the past few decades, with decreases in coral cover by more than 50% since the 1970s (Jackson et al., 2014) and 90% of the region's remaining reef systems being classified as threatened (Plaisance et al., 2011).

Given the ecological and economic importance of coral reefs, new approaches are needed to sustain their ecosystem function. Reef restoration aims to facilitate the recovery of damaged or degraded coral reef ecosystems that are unable to do so naturally (Hobbs and Cramer, 2008). While Marine Protected Areas are needed to support fish diversity and trophic structure, their establishment is not always sufficient to ensure coral reef recovery in the face of increasing threats and subsequent reef degradation (Cox et al., 2017). There is an urgent need for effective methods to strategically restore and augment the recovery of coral reefs and the ecosystem functions they provide (Boström-Einarsson et al., 2020). Coral restoration is a relatively new field and efforts in the Caribbean have focused on the recovery of endangered coral populations (e.g., *Acropora palmata* and *A. cervicornis*) and are increasingly expanding to restore the structure and function of coral communities and ecosystems (Boström-Einarsson et al., 2020; Bayraktarov et al., 2020). These restoration activities are driving an increased environmental stewardship awareness and community-based interest and action in protecting coral reefs (dela Cruz et al., 2015; Chamberland et al., 2017).

One significant challenge of coral restoration has been the selection of sites that will provide the best chance of restoration success (Foo and Asner, 2019). For years, the need for the selection of suitable outplant sites based on logistical, ecological, and physical factors that are conducive to coral survival has been acknowledged (Hernández-Delgado et al., 2014). Commonly, *A. cervicornis* restoration efforts in the Caribbean identify outplant sites by considering a wide array of factors, including logistical factors (e.g., distance from nursery and accessibility), ecological factors (coral cover, macroalgae cover, herbivore, and predator abundance), and physical factors (depth, water quality, temperature, and water flow) (Johnson et al., 2011; Hernández-Delgado et al., 2014, Mercado-Molina et al., 2015; Ladd et al., 2018). However, site selection has been traditionally accomplished via SCUBA-based surveys, which covers a limited portion of the potential habitat available to be restored and can be time and resource intensive. Additionally, there is an increasing call for coral reef restoration efforts to incorporate considerations of climate change and resilience characteristics, including indicators such as connectivity, biodiversity, and temperature variability (McClanahan et al., 2012; Shaver et al., 2020). Advancements in remote sensing provide important and novel opportunities for coral reef restoration site selection, including locating new sites, reducing in-water time to find sites, and the ability to incorporate key resilience indicators and climate change projections into restoration to target efforts that maximize coral success and the scalability of outplanting efforts (Foo and Asner, 2020).

Recent advances in coral science are providing exciting new methods to support reef restoration at broader scales (Boström-Einarsson et al., 2020). However, these methods and technologies must be integrated, tested, and applied at adequate scales to make a demonstrable impact on coral reef ecosystem recovery. Additionally, the rapid pace of reef degradation demands proactive collaboration and knowledge sharing among conservation organizations, scientists, governments, for-profit companies, and community stakeholders in the Caribbean and globally. The cost of coral restoration has been estimated from $1,717 up to $2,879,773 USD per hectare (Bayraktarov et al., 2016), and some research suggests that in certain contexts, current costs outweigh benefits (De Groot et al., 2013). Remotely sensed data can be economically incorporated into restoration activities because high spatial and spectral resolution data can be collected from airborne sensors over large areas (thousands to millions of hectares) with costs of ∼$0.01 USD per hectare at non-profit rates (Asner et al., 2014). Thus, the use of remote sensing for selecting suitable coral restoration outplant sites provides a cost-effective way to increase restoration success across large areas, particularly when multiple government agencies or other entities combine resources to map multiple project areas during a single field campaign.

With the purpose of integrating new remote sensing technologies into the strategic selection of coral reef restoration sites, The Nature Conservancy (TNC) partnered with the Asner Lab of Arizona State University to collect high resolution imaging spectrometer data over the southeast Dominican Republic in May 2017 using the Global Airborne Observatory (GAO) (Asner et al., 2012). The area mapped is part of the Santuario Marino Arrecifes del Sureste (SMASE), the second largest protected area in the Dominican Republic, where intensifying tourism activities increasingly threaten vulnerable marine ecosystems. SMASE has a co-management arrangement contained in a 25-year agreement with the Ministry of Environment and Natural Resources and a unique public-private partnership among local and international institutions, including Fundacion Grupo Puntacana (FGPC), Fundacion Dominicana para los Estudios Marinos (FUNDEMAR) and TNC. As part of the co-management of SMASE, these institutions, among other local partners, organize coral outplant events in which local stakeholders (NGOs, diving operators, tourism sector, and government agencies) engage in multi-day coral restoration efforts both within and around the sanctuary and in nearby reefs. Known as "*Coral Manias*," these activities have facilitated the successful outplanting of thousands of *A. cervicornis* fragments onto degraded reefs throughout tens of square kilometers of coastal areas following repeated community events. Bávaro is a coastal community in northern Punta Cana, just 3 kilometers west of the SMASE boundary (**Figure 1**), that was identified as a high priority restoration site due to its degrading reefs and need for coastal protection.

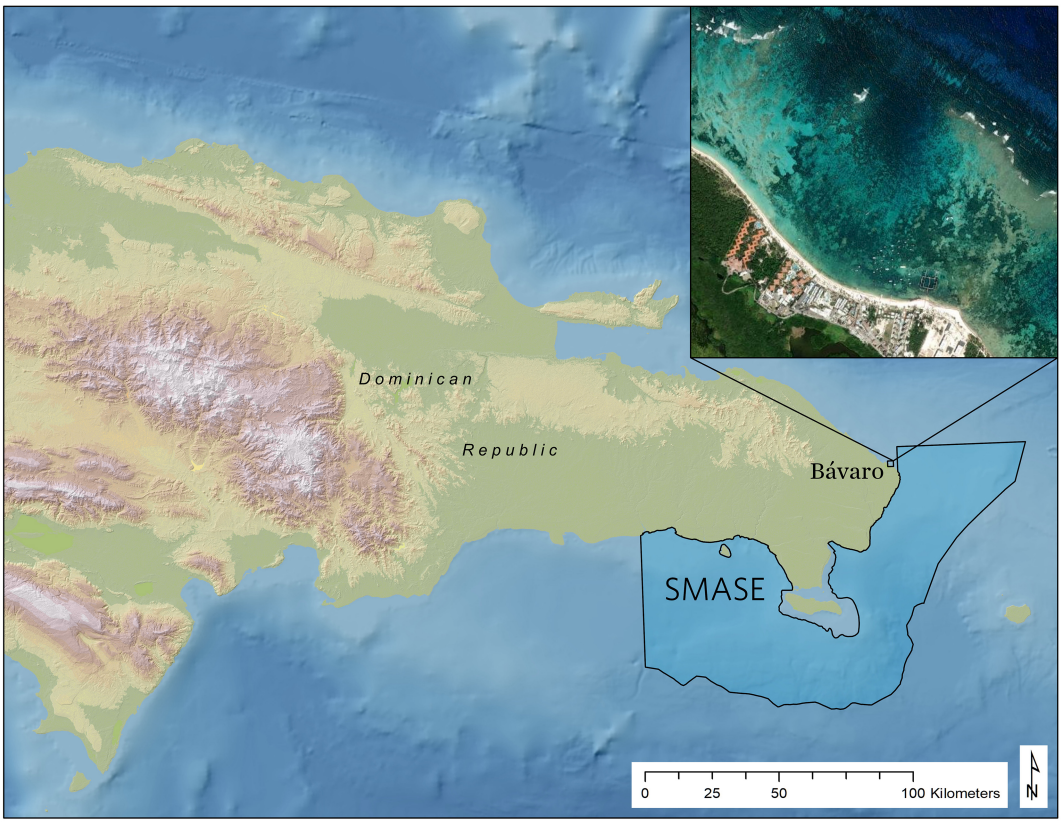

FIGURE 1 | Reference map showing the location of the outplant locations in Bávaro near the South East Marine Sanctuary (SMASE: Santuario Marino Arrecifes del Sureste), the second largest protected area in the Dominican Republic where the Global Airborne Observatory (GAO) acquired imaging spectrometer data in May 2018.

In the past, outplant sites for these events have been selected based on local expertise, without specific and/or standardized criteria. Our objective is to present a case study that uses GAO-derived map products within a suitability model framework to provide a quantitative and replicable method for selecting coral restoration sites with the goal of increasing outplant survival over time.

MATERIALS AND METHODS

Remote Sensing Data Collection

From May 1, 2018 to May 25, 2018, the GAO operated in the Dominican Republic, collecting aerial imaging spectrometer data using a high-fidelity visible-to-shortwave infrared (VSWIR) imaging spectrometer and a dual-beam light detection and ranging (LiDAR) scanner. Full descriptions of the aircraft, instrumentation, and data processing are provided by Asner et al. (2012, 2020a). A position and orientation system (POS) enabled the computation of aircraft position to within 5 cm (RMSE: root mean squared error) and aircraft orientation for the duration of all flights. GPS timing data were collected during flight by all three instruments, and this information, along with known position and instrument boresight offsets, allowed precise back-computation of position and orientation for the receiver of each instrument at all times during flight.

Collection location could change during each flight day and was actively managed based on need, cloud cover, and windspeeds to provide both the most efficient use of time and the best conditions for spectroscopic seafloor measurements. To maximize data consistency, airborne operations were performed from 0830 to 1100 local time. During flight, instrument settings were set for the planned nominal flight altitude of 1 km above the sea surface. Flight lines were spaced to achieve 50% overlap in VSWIR spectrometer coverage. Aircraft groundspeed was maintained between 130 and 140 kt. LiDAR pulse frequency was set to 200 kHz (100 kHz per laser), and scan frequency was 34 Hz with a field of view of 38°, allowing 2° of buffer on each side of the spectrometer field of view of 34°, and achieving a nominal pulse density (over land) of more than 8 pulses m^{-2}.

Field Data Collection

A field campaign to measure and characterize the project area was conducted during the same time as the GAO data collection. *In situ* data collected for calibration and verification included 122 GPS-referenced underwater video transects, 37,400 transducer bathymetric points, and 2,477 benthic digital photos collected at sub-meter accuracy. High resolution satellite imagery converted

to MBTile format and loaded into the Locus Pro application on a tablet was used to identify and navigate to survey point locations representing various benthic habitat types. A SeaViewer Sea-Drop 6000 HD (Tampa, FL, United States) underwater video camera with a 30 m vertical cable was used to record video transects at 1–2 m above the seafloor. A GPS reference point was collected at the start and end of each transect to allow for georeferencing of video surveys. A total of 152 km of bathymetric field measurements were collected using a Lowrance Elite7Ti® system with a xSonic P319 (50/200kHz) transducer and 10Hz GPS receiver that measured continuous depth readings at 3 pts/sec. In areas inaccessible by boat, snorkeler-based transect surveys were swam using a GoPro Hero 6 camera capturing video footage of various benthic habitat types. In addition, very high resolution (3 cm pixel) orthophoto mosaics and digital surface models were acquired for selected candidate coral outplant locations using a DGI Phantom 4 Pro Unmanned Aerial Vehicle (UAV).

To calibrate the imaging spectrometer benthic classification (e.g., percent live coral and algal cover), underwater photos were collected with coincident highly accurate positional data acquired with a survey grade Trimble Global Navigation Satellite System (GNSS) receiver. The GNSS receiver antenna was mounted to the top of a foam bodyboard and a Sony a6300 camera was positioned in a waterproof housing directly below this antenna on the underside of the board to collect vertical photos of the seafloor (**Figure 2**). The board was towed using a nylon tether rope. Cabling from the camera (both an HDMI and USB) and the GNSS was long enough to be included next to the tether. An operator on the boat was then able to view the camera's viewfinder in real time using the video feed from the HDMI cable. The USB cable allowed for triggering of the camera shutter on an ad-hoc basis. At the same time, the operator could also trigger the GNSS system to record a point as that receiver was also onboard (in practice this was often done by a second operator). In addition, the GNSS was set to continuously log at 5 Hz (5 times per second).

Cellular or other internet connection on the boat was not reliable, thus the data were not corrected in real time. Therefore, it was necessary to post-process the GNSS locations and then match the images to those positions. Base station data from the Continuously Operating Reference Stations (CORS) sites, established by the National Geodetic Survey (NGS), and were used to correct the data using Post Processing Kinematic (PPK) techniques[1]. The Trimble Pathfinder Office software was used

[1]https://www.ngs.noaa.gov/CORS/

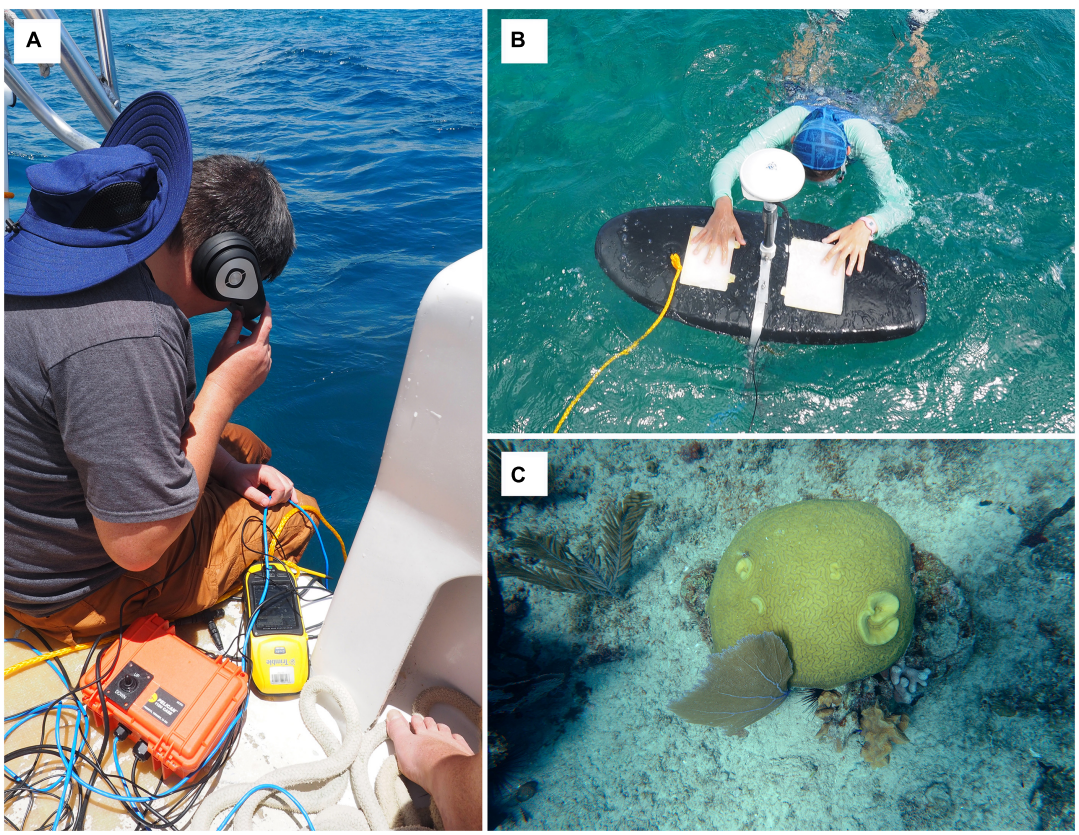

FIGURE 2 | Field data collection used to calibrate the imaging spectrometer benthic classification: **(A)** an operator on the boat is able to view the video feed from the camera in real-time via HDMI cable; **(B)** the GNSS receiver antenna mounted to the top of a foam bodyboard and a Sony a6300 camera positioned in a waterproof housing directly below this antenna on the underside of the board to collect vertical photos of the seafloor; **(C)** an example of one of the 2,477 underwater photos collected with <1 m positional accuracy that was later classified by proportional cover in the following benthic classes: sand, live coral, algae, seagrass, and rubble.

to post-process the corrections. Once the field-collected GNSS rover files are supplied to the software, the necessary base station files that match the specific time period in which the rover files were collected are downloaded, and the correction is performed. Trimble Pathfinder Office reported a final horizontal accuracy under 1 m for >98% of the locations with the majority being under 100 mm. Photos were assigned a location based on their timestamp to the closest corrected GNSS position in time. The few points with time differences over 1 s or with reported accuracies over 1 m were discarded. A total of 2,477 photos with a precise GNSS location (<1 m) were classified into their respective percent classes (sand, live coral, algae, seagrass, and rubble), and a point feature class with the assigned classification was created and used as ground reference for extracting these classes from the imaging spectroscopy data.

Data Processing

Airborne data from all three instruments were processed for orthorectification as well as radiometric and atmospheric correction. The raw LiDAR point cloud data were converted to a 50 cm resolution digital surface model (DSM) by interpolating between the first returns from each pulse using the GDAL writer functionality of the Point Data Abstraction Library (PDAL Contributors 2018). Regions missing from this surface map, due to specular reflection of the LiDAR beams off of the water surface, were filled in using inverse distance weighted interpolation (IDW) with power 2.

With the LiDAR DSM and known position, orientation, and camera lens model for each instrument, the 3-dimensional position of each spectrometer and digital camera pixel was ray-traced to the sea surface level. The raw spectrometer data collected onboard the GAO were first converted to radiance using laboratory calibration data collected before the campaign. The radiance data contain 427 spectral channels covering the wavelengths between 350 and 2500 nm in 5 nm increments. Using the LIDAR-derived observation angles and elevation as inputs, atmospheric correction was performed with a modified version of the ATREM model (Thompson et al., 2017).

The orthorectification for each flight line was adjusted for water refraction and depth. A neural network deep learning model was used to compute depth for each flight line (Asner et al., 2020a). Then for each spectrometer pixel, the at-surface view zenith angle, φ_a, was modified for refraction at the air-water interface to get below-surface zenith angle, φ_b, using Snell's law and standard refractive indices of 1.33 and 1.00029 for water and air, respectively. Together, this resulted in the conversion equation: $\varphi_b = \sin^{-1}(0.752098 \sin \varphi_a)$. From the original sea surface location, this angle was traced to the modeled ocean floor depth to get a new 3-dimensional position for each spectrometer pixel representing the pixel location on the seafloor.

With the location of each spectrometer pixel known, individual flight lines were mosaicked together using a strategy of minimum glint, where glint is defined as the average reflectance value for the five spectral bands covering the wavelengths 890–910 nm for each pixel. For each mosaic map pixel location, data from the flight line with the lowest glint at that location was kept.

Bottom Surface Reflectance

Water scatters higher energy wavelengths of light and absorbs lower energy wavelengths, making the shape and brightness of the spectrum observed at the surface of the water strongly dependent upon the depth of the water. To account for this in the spectrometer data, the bottom reflectance spectrum was estimated using an empirical approach with two assumptions: (1) sufficiently constant reflectance spectrum of sand patches within the region of interest, and (2) minimal change in inherent optical properties of water within the region of interest. The algorithm to back-compute bottom reflectance consisted of the following steps:

1. Identify individual pure sand patches within the region of interest, ensuring to select patches from the full range of depth in the region of interest.
2. Obtain a bottom reflectance spectrum that will represent pure sand, $b_{sand,*}$.
3. Collect surface reflectance spectra for all pixels within the identified sand patches, r_*.
4. For each wavelength in the collected reflectance data, fit a loess model of power 1 (Cleveland and Devlin, 1988) to the brightness of that wavelength against depth and store this model.
5. To compute bottom reflectance for each pixel, get the predicted surface reflectance for sand, $\hat{r}_i$, at the given depth using the models fit above. Then bottom reflectance, $\hat{b}_i$, is computed as:

$$\hat{b}_i = b_{sand,i} \frac{r_i}{\hat{r}_i}$$

The Classification Model

Training data for the deep learning model were built from a combination of 2,477 GPS subsurface photos as well as 633 hand-selected pure class locations identified using the GAO 3-band color orthorectified surface reflectance imagery. Each of the subsurface photos were evaluated to estimate proportional cover in the following benthic classes: sand, live coral, algae, seagrass, and rubble. The hand-selected points were sand, seagrass, and "other" which was used as a catch-all class to help the model correctly remove pixels associated white water, unfiltered sun glint, dark shadows, and surface features such as floating sargassum. For each training sample (pixel), the 57-band modeled bottom surface reflectance was retained along with the modeled depth at that location.

The TensorFlow package in Python (Abadi et al., 2016) was used to train a feed-forward neural network (NN) model to predict the proportional class membership of each pixel using the 57-band estimated bottom reflectance spectrum as well as the matching GAO depth as input. Eighty percent of the samples were selected at random for use in model training, 10% for checking optimization stopping criteria, and 10% for validation. The model architecture included a 58-node input layer, along with four dense hidden layers of 500 nodes each. All dense hidden layers used a RELU activation function. Finally, a six-node output layer was used, one each for proportion of sand, live coral cover, algal cover, seagrass cover, rubble cover, and "other," using a linear activation function. Here, algal cover is defined as a combination

of strong light-absorbing turf algae and weaker light-absorbing macroalgal cover. Mean square error (MSE) was used as the loss function. The ADAM optimization algorithm (Kingma and Ba, 2014) was used to fit the network coefficients to the training data, with an automatic stop determined as no improvement in the validation set loss value in 30 epochs. The model required 103 epochs to reach this optimization criterion, with the overall unweighted MSE across all classes reaching 0.0275. The accuracy of these data and methods have been previously demonstrated in Asner et al. (2020b) where airborne estimates of live coral cover were found to be highly correlated with field-based estimates of live coral cover ($R^2 = 0.94$).

Maps for Outplanting Strategy

With the trained classification model, maps of modeled proportion of sand, live coral, algae, and seagrass cover were produced for each 1 × 1 m pixel in the GAO coverage of the outplanting region with an estimated depth of <16 m. In addition to the proportional cover maps, two additional maps were prepared for assisting outplanting strategy. First, a map of benthic depth was built using the GAO depth model defined in Asner et al. (2020a). Second, habitat complexity was modeled from the benthic depth map using an algorithm for surface complexity defined in Jenness (2004), with a window size of 9 pixels × 9 pixels (9.0 m × 9.0 m). Because the distribution of raw complexity values is extremely skewed to the right, an empirical probability integral transformation was used to rescale the values to follow a uniform distribution and range from 0 to 1. For this procedure, the transformed complexity values are computed as the rank of the original complexity value divided by the number of pixels. Spatial boundaries for geomorphic zones (e.g., fore reef, reef crest, back reef, and lagoon) were manually digitized based on image interpretation of high resolution (1 m) satellite image base maps available in Google Earth, Microsoft Bing, and Esri and were used as selection criteria to supplement the GAO data layers.

Outplanting Criteria

Potential sites for outplanting in Bávaro were chosen based on two types of criteria: (1) logistical and (2) ecological. Logistical criteria aimed to facilitate outplanting by reducing time and costs in the field. Logistical criteria included distance from the nursery site (<1000 m) and wave exposure. Back reef habitats were targeted to avoid strong currents and surge, making the outplanting process safer for volunteers and potentially lowering the probability of coral fragments detaching from the substrate. Ecological criteria aimed to enhance the probability of survivorship of outplants were selected. Criteria ranges were based on what is known about the ecology and biology of *A. cervicornis* and the local and regional restoration efforts that have been conducted for the species in the past (Hernández-Delgado et al., 2014; Mercado-Molina et al., 2015; Ladd et al., 2018). Ideal ecological criteria were identified: (1) depth: 3–7 m; (2) maximize percentage of live coral: >2% (highest in this area was 10%); (3) minimize percentage of algal cover: <80%; and (4) maximize habitat complexity: >0.3 (**Figure 3**). These ranges were based on local conditions in the Bávaro area (i.e., <30% algal cover or higher live coral cover would be more ideal conditions but was not possible within the area of interest). The GAO-derived data layers were filtered to these ranges and subsequently intersected to identify areas that met all criteria. We also included information on the coastal protection and vulnerability reduction benefits of reef locations, as modeled using the InVEST (Integrated Valuation of Ecosystem Services and Tradeoffs) software, which uses geophysical and natural habitat characteristics of coastal landscapes to compare their exposure to erosion and flooding in severe weather (Sharp et al., 2018; Harris et al., 2018). When all criteria were considered, approximately 50,900 m^2 of potential suitable area was selected for outplanting activities.

Community Outplant Event

Overall, the Bávaro outplanting area showed clear signs of old reef degradation, which is common across backreefs habitats in Bávaro. However, visual inspection of the pre-selected area from satellite imagery and field visits confirmed these sites were suitable for outplanting corals and met the selection criteria with high accuracy. More specifically, the area was composed of patchy hard bottom habitats with crevices, desired levels of rugosity, and sufficient percentages of live coral and algal cover (**Figure 4**). Selected sites were comprised of scattered pinnacles (5–10m depth) distributed across sandy bottoms, which is common for back reef habitats. Only a few coral species were observed, largely small-size brooders such as Agariciid (*Agaricia tenuifolia* and *A. agaricites*) and Poritids (*Porites porites* and *P. astreoides*), whereas large reef builders such as *Orbicella annularis*, *O. faveolata*, *Colpophyllia natans* and *Pesudodiploria strigosa* were less common. Algae clearly dominated the reefs, with species in the genera *Dyctiota* spp. and *Lobophora* spp. being the most prevalent.

Snorkelers field-checked the identified area of interest to verify the site met all relevant criteria and was suitable for coral outplanting. A total of 1,722 fragments of *A. cervicornis* ranging from 100 to 160 mm (mean 123 mm 32 SD) were outplanted at this location during a community-based citizen science outplant event, "*Coral Mania*," hosted by Consorcio Dominicana de Restauracion Costera, Grupo Punta Cana Fundación, FUNDEMAR, and Counterpart International between November 17 and 21, 2019. More than 30 volunteer divers in three boats worked to outplant corals at nine plots: "*Coral Mania 1*" (CM1), CM2, CM3, CM4, CM5, CM6, CM7, CM8, and CM9 (**Table 1**). Prior to outplanting, divers enhanced the substrate by removing algae from the location where each coral fragment was to be attached. This process was minimal since the model had previously selected these sites based on lower levels of macroalgal cover. During the outplanting phase, divers outplanted in areas with low frequency of sessile organisms which compete with corals for substrate space (e.g., drilling and/or encrusting sponge, hydrocorals, etc.) and by the large numbers of crevices and overhangs that provide rugosity to the habitat.

Outplant Monitoring

Indicators of coral outplanting success include survivorship, growth rates, and community structure. Coral fragments and

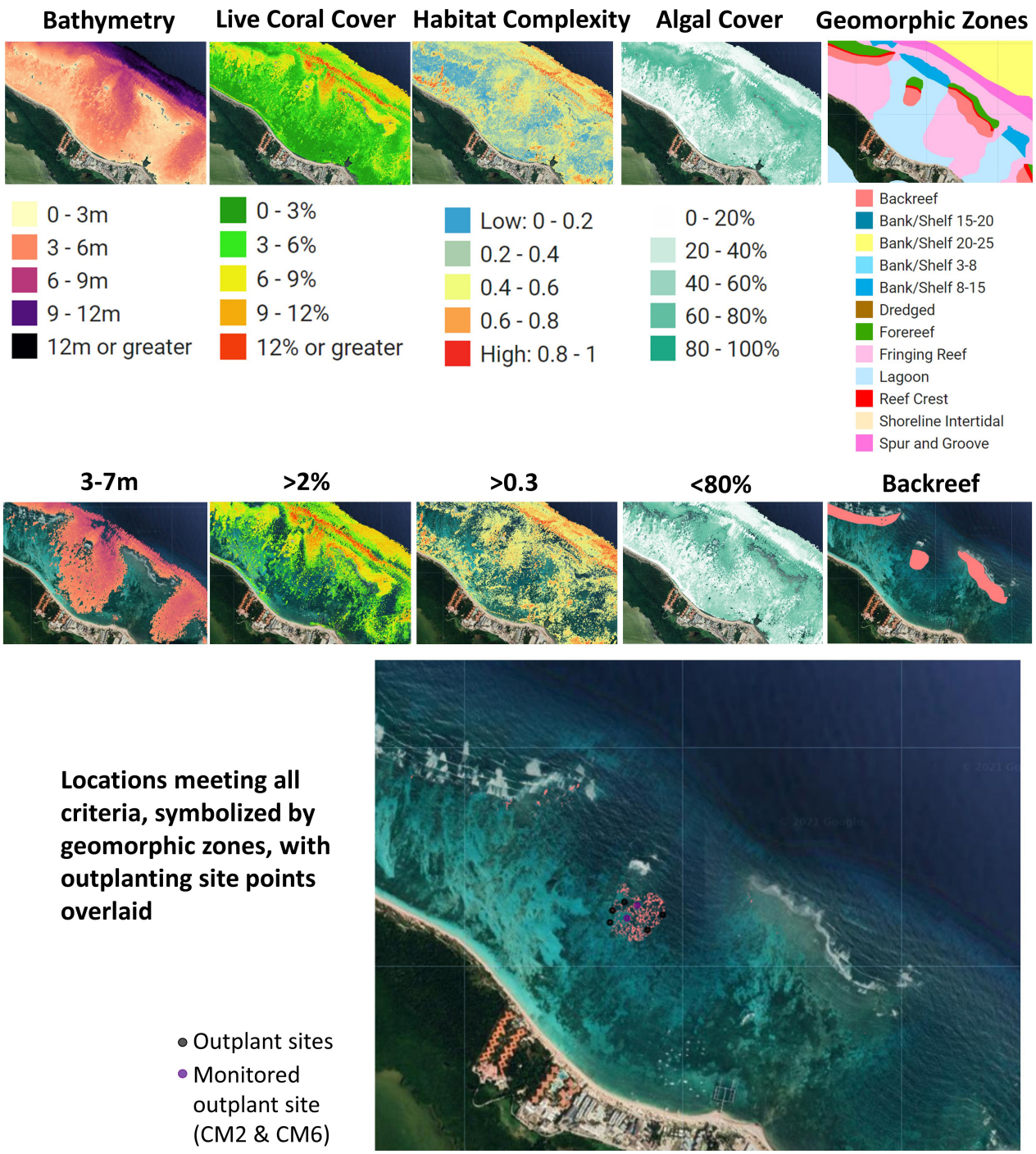

FIGURE 3 | Data layers and criteria used to select suitable outplant sites in Bávaro, Dominican Republic. Sites were identified using the Coral Outplanting Siting Guide Google Earth Engine (GEE) app and used as the focal restoration area in the community-led outplanting activity. These criteria were based on key ecological factors of *Acropora cervicornis* that would promote the likelihood of outplant survivorship.

the associated benthic community structure were monitored at four randomly selected outplant sites (CM1, CM2, CM3, and CM4) during January 2020 before the COVID-19 pandemic and again in October 2020 when diving restrictions were lifted. During these surveys, 31–50 and 16–41 individual fragments were photographed in January and October 2020, respectively, using a 300 mm ruler as reference to determine the size of each fragment and the percent living tissue. Coral fragment size was estimated using measurements of maximum length and width of the individual coral colony using ImageJ software. Growth

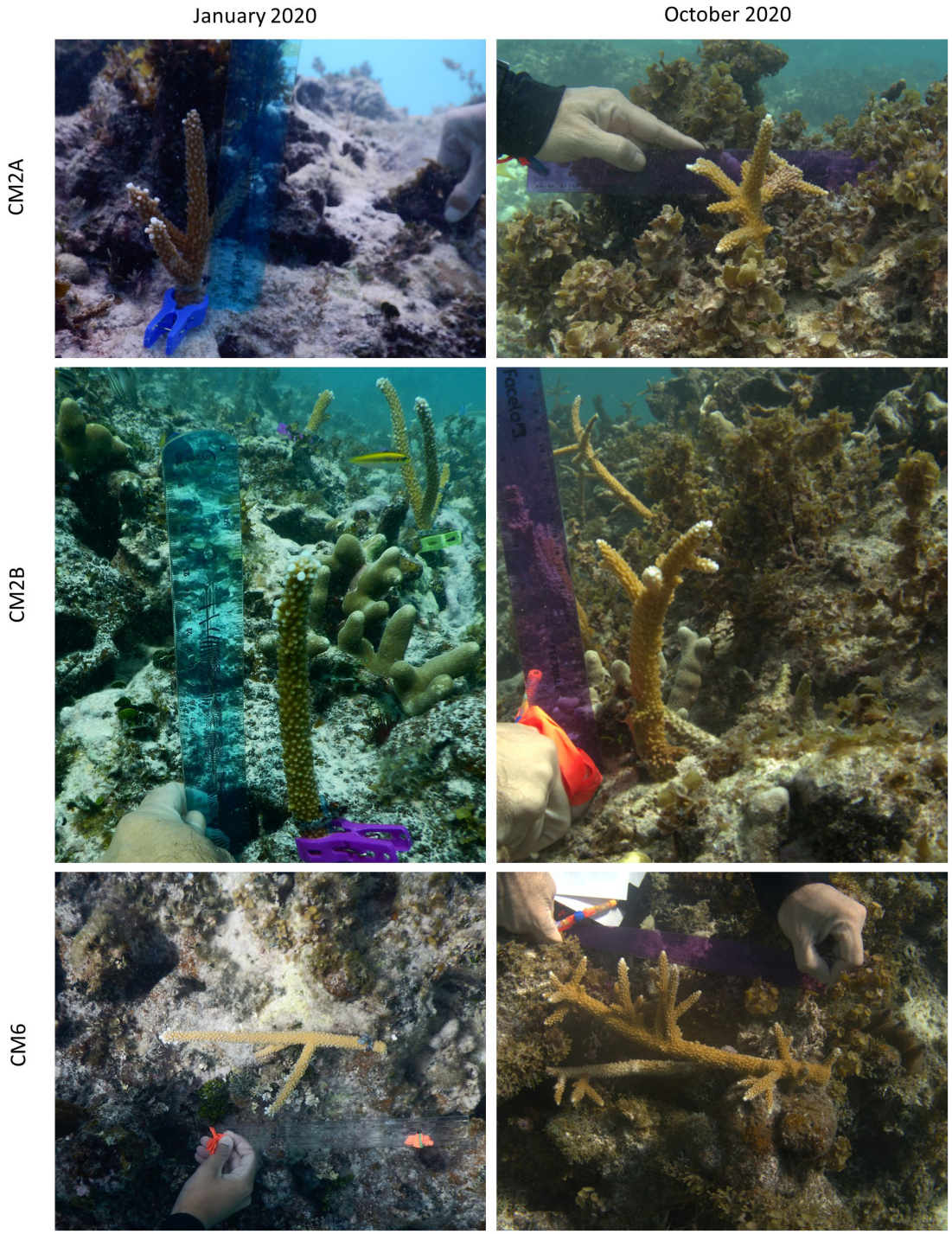

FIGURE 4 | Coral monitoring photos comparing growth of outplants between January 2020 and October 2020.

rates for the population of outplanted corals were calculated as the difference between the estimated mean of coral colonies in January and October 2020 and the estimated mean of the initial coral size at the time of outplanting (November 2019). Coral colony survivorship was measured as the change in the number of living coral colonies within the plots, while percent live coral tissue per colony was visually estimated to the nearest 5% using photos in ImageJ. While coral fragments were only monitored three times in 1 year, both survival and growth rates were measured with high precision from large sample sizes.

In addition, 7 to 9 photos of the benthic substrate were taken approximately 2m above the substrate in three out of

TABLE 1 | Locations and GAO values of the outplant sites selected.

Site	Depth (m)	Live coral cover (%)	Habitat complexity	Algal cover (%)	Geomorphic zone	Seagrass cover (%)	Sand cover (%)
CM1*	3.67	1.67	0.28	12.66	Backreef	7.59	57.91
CM2*	3.91	1.54	0.34	65.21	Backreef	1.19	17.42
CM3*	3.91	1.54	0.34	65.21	Backreef	1.19	17.42
CM4*	3.91	1.54	0.34	65.21	Backreef	1.19	17.42
CM5	2.90	4.27	0.44	79.01	Backreef	0.56	1.86
CM6	5.04	1.00	0.23	16.79	Backreef	8.23	70.80
CM7	2.62	3.89	0.46	78.29	Backreef	0.62	3.56
CM8	4.17	1.87	0.41	58.46	Backreef	3.60	16.43
CM9	4.85	0.68	0.58	73.48	Backreef	3.41	6.43

**Monitoring sites.*

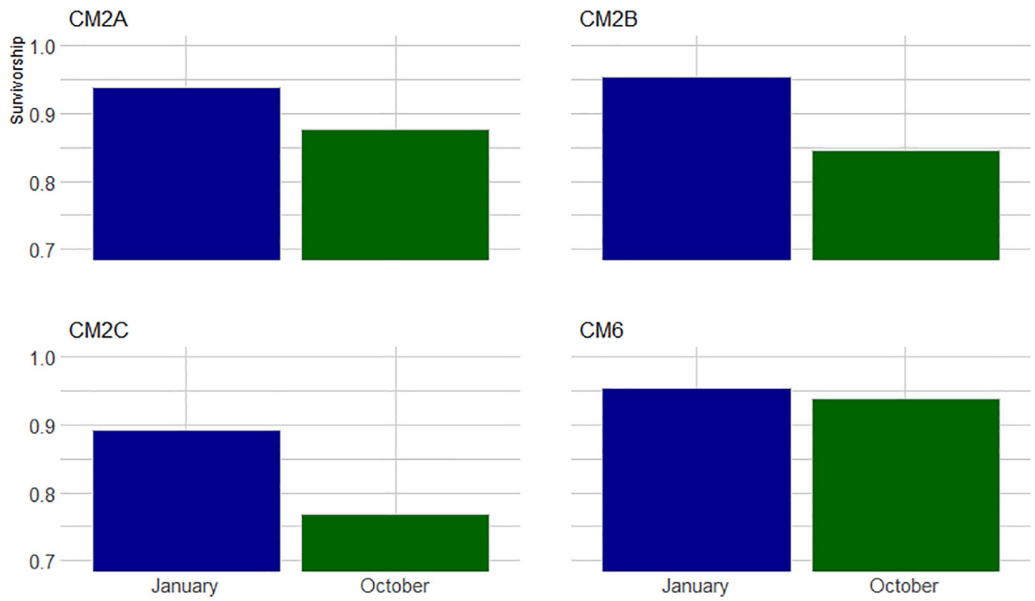

FIGURE 5 | Proportion of colonies that survived between November 2019 to January 2020 and October 2020 across four monitored sites at Bávaro.

four randomly selected sites to determine the percentage of live cover of selected major taxonomic groups [i.e., scleractinian corals, hydrocorals, octocorals, sponge, turf algae, macroalgae, and crustose coralline algae (CCA)] and abiotic substrates (i.e., bare substrate, dead coral, sand, and rubble). We analyzed 25 random points within each photo using the software photoQuad (Trygonis and Sini, 2012) following procedures outlined in the Global Coral Reef Monitoring Network protocol (Miyazawa et al., 2020). Benthic community changes were monitored in January 2020 but could not be assessed in October 2020 due to challenges caused by the COVID-19 pandemic.

RESULTS

Survivorship and Growth

In January 2020, 2 months after the outplant event, survivorship among the four subplots over a random sample of 65 coral fragments was 91.6% ± 4.7 SD. By October 2020, almost a year after the outplant event, survivorship remained above 76% with an average of 83.5 ± 4.7 SD (**Figure 5**). During the first monitoring event in January 2020, the majority of outplants had 100% living tissue; however, by October 2020, the coral fragments had lost 26–84% live tissue, likely due to predation (**Figures 6**, **7**). Moreover, results showed that the size of the coral fragments increased 0.5 to 3-fold with an average initial size 123 mm ± 32 SD and an average size after 3 months of 256 mm ± 15.3 SD. During the October 2020 revisit, continued growth was observed, but at a slower pace, with the exception of site CM1 where coral fragments doubled in size. Size frequency distributions, estimated in January 2020, indicate that 70–90% of the coral fragments had grown to reach sizes of 250–500 mm, further indicating rapid growth rates for the population of outplanted corals (20–40 mm per month). In October 2020, the size frequency distribution only changed at site CM1, suggesting that corals at this site continued to grow at similar rates that were observed after the first 3 months (**Figure 8**).

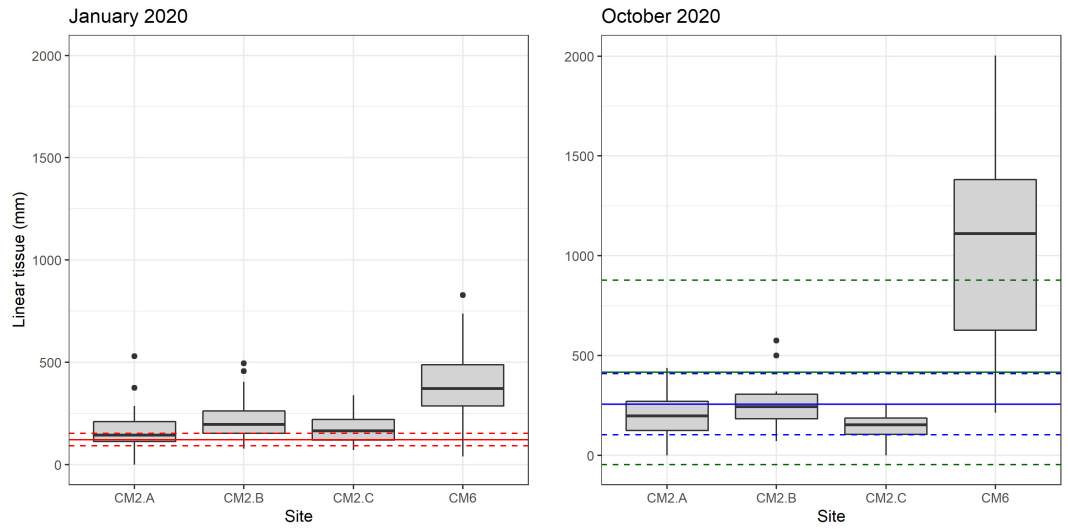

FIGURE 6 | Linear growth box plot for colonies that survived between January 2020 and October 2020 across monitored sites. Solid and dashed red lines in panel January 2020 indicate the average and confidence interval (CI 95%) of initial sizes at the moment of outplanting (November 2019), respectively. Solid and blue dash lines in panel October 2020 indicate average and confidence interval (CI 95%) of growth recorded in January 2020. Solid and dashed green lines indicate mean and confidence interval (CI 95) recorded in October 2020, respectively. Black dots represent outliers.

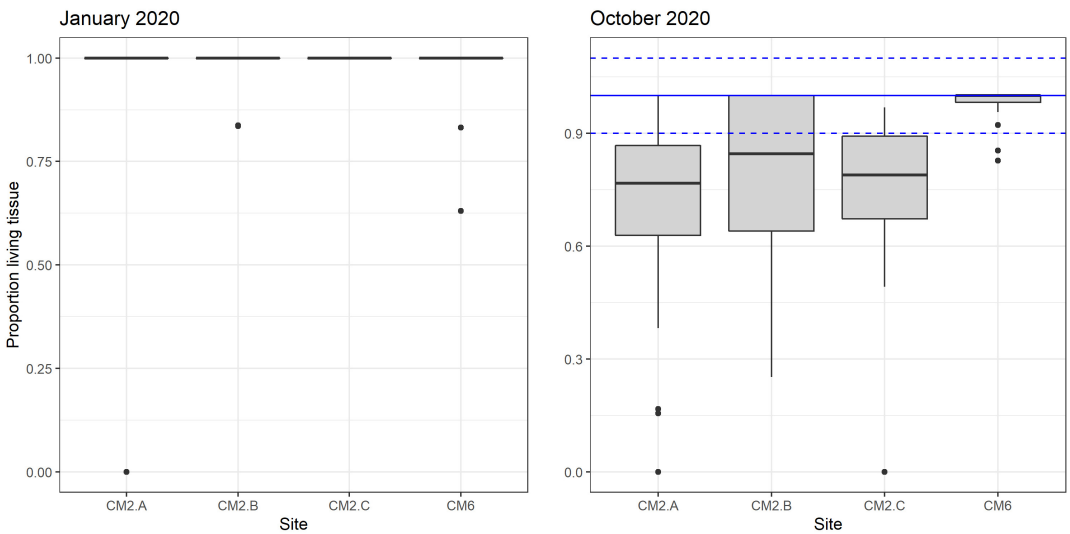

FIGURE 7 | Proportion of living tissue box plot remaining on corals outplanted across sites between January and October 2020.

Benthic Community

A total of 3 months after outplanting the corals, the benthic community structure remained consistent with the findings observed in the site selection criteria model. Coral cover ranged from 3.5 to 4% with maximum values of 8–10% and an average value of 3.6% ± 0.4 SD. Turf algae was the predominant substrate, ranging from 34 to 46 % with an average of 40.9 % ± 5.9 SD, followed by the macroalgae, which varied from 27 to 33% with an average of 29.6 % ± 3.2 SD. CCA seldom exceeded 10%, varying from 4.7 to 6.4% with a mean of 5.4% ± 0.7 SD. Sponges and other substrate competitors were rare across the monitored sites, seldom exceeding 3% of cover (**Table 2**).

DISCUSSION

During the past few decades, restoration efforts have increased significantly as coral reefs continue to decline (Boström-Einarsson et al., 2020). Coral restoration is a complex task which must consider multiple interactive factors (Hernández-Delgado et al., 2014) with the acknowledgment there are a

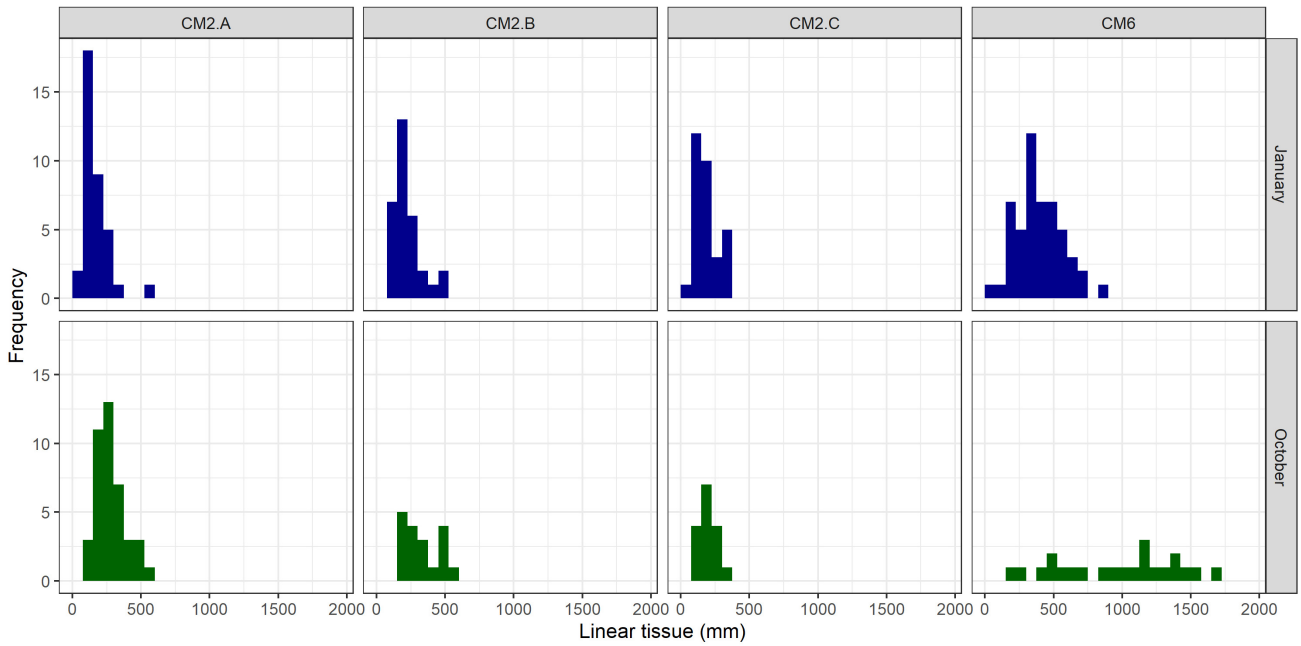

FIGURE 8 | Size frequency distribution of colonies per monitoring site 3 months (January 2020) and 11 months (October 2020) after the outplant event (November 2019).

TABLE 2 | Coral monitoring: benthos analysis (January 2020).

Site	Benthic group													
	Scleractinian corals		Sponges		Macroalgae		Turf		Crustose coralline algae		Sand		Other	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CM3	3.56	3.00	3.56	3.71	28.89	10.91	34.22	13.28	4.78	4.63	20.44	6.77	5.22	6.12
CM4	4.14	4.63	0.57	1.51	26.46	11.01	39.86	13.93	6.14	5.36	12.57	8.14	10.29	7.25
CM1	3.29	2.75	0.57	1.51	33.14	15.61	39.14	12.75	5.14	3.76	9.14	7.90	9.14	5.98

variety of ecological and biophysical processes that cannot be detected using remotely sensed data. The reasons for coral decline vary from site to site and the costs and logistical constraints make it difficult to conduct restoration at large and ecologically relevant spatial scales. However, identifying strategies to increase the scale of restoration efforts is a priority for restoration practitioners and scientists (Boström-Einarsson et al., 2020). The development of science-based solutions for analyzing and optimizing the selection of suitable sites for outplanting corals across large reef sections is a necessary step for achieving this goal.

We address several ecological and logistical criteria for outplant site selection and demonstrate a proof-of-concept that remote sensing and suitability modeling can be used to increase the efficiency of coral outplant site selection. The approach we present is the first time this technology has been used to guide restoration practitioners in restoration site selection for a coral outplanting event implemented by community practitioners and citizen scientists. Our methods permit the screening of large areas (km^2) for identifying a variety of suitable outplanting habitat locations across broad scales that otherwise would not be possible using traditional finer-scale site selection methods such as SCUBA–based surveys. The results of our coral survivorship surveys exceed 76% after 11 months, suggesting the technology and methodology we present provides an effective approach in identifying suitable sites where corals are more likely to persist. We suggest additional research is needed in a variety of coral reef conditions and locations to further test and refine this approach for broader use and application in the field of coral reef restoration.

Our methods are replicable and accessible via a web app where restoration practitioners can access the criteria modeling framework and datasets for pilot sites in the Dominican Republic and U.S. Virgin Islands. The *Coral Outplanting Siting Guide*, a Google Earth Engine (GEE) app, provides coral managers and restoration practitioners with the ability to repeat the suitability modeling process to dynamically select outplant locations based

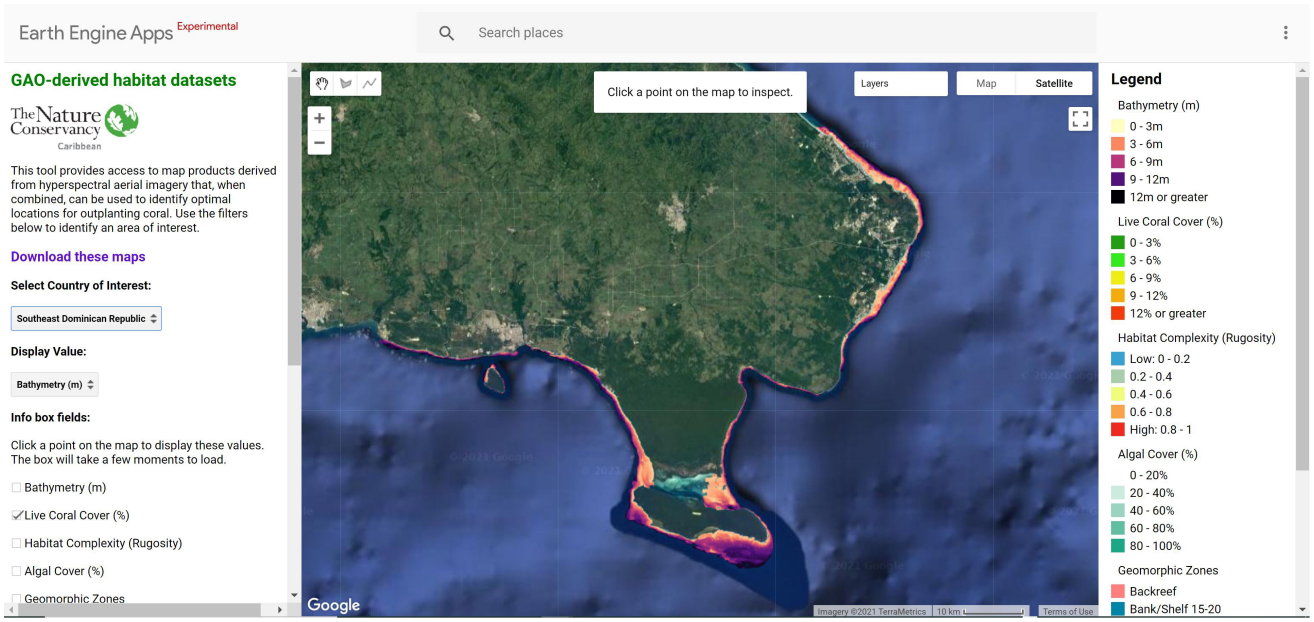

FIGURE 9 | The Coral Outplanting Siting Guide, a Google Earth Engine (GEE) app, an open-source web browser-based tool that provides coral managers the ability to dynamically model and select outplanting locations based on site-specific or coral species-specific criteria. The tool allows users to interact with large spatial datasets without requiring software installation, technical knowledge, or data storage capacity.

on site-specific or coral species-specific filters[2] (**Figure 9**). Our approach provides seven criteria that can be used for filtering the map to select a specific outplant site: bathymetry (m), live coral cover (%), habitat complexity (rugosity), algal cover (%), geomorphic zone, seagrass cover (%), and sand cover (%). The user can input custom ranges (e.g., 3–7 m depth, 10–80% live coral cover) and results display only areas that meet those criteria. Alternatively, criteria values at existing successful outplant sites can be identified using the latitude and longitude of the site. Measurement tools can be used to select outplant sites based on logistical criteria including distance to a coral nursery lab or total area required for an outplant site.

While site selection is key for success and is extremely valuable for scaling up these efforts (Foo and Asner, 2019), a more complete understanding of coral species biology, and ecology is fundamental for successful coral restoration. Coral outplant survivorship not only depends on habitat suitability and the features of the physical environment (Shaver and Silliman, 2017, Ladd et al., 2018), but is also determined by a series of biological process such as predation (Glynn, 1962; Baums et al., 2003; Miller et al., 2014), herbivory (Burkepile and Hay, 2010; Shaver and Silliman., 2017; Ladd et al., 2018; Lefcheck et al., 2019; Cano et al., 2021), disease (Hernández-Delgado et al., 2014), and genetic identity of coral outplants (Drury et al., 2017). These types of variables can vary greatly across space and time and are undetectable in remotely sensed images. Thus, we recommend outplant site selection should be evaluated in combination with remotely sensed data and field-based surveys where ecological processes and site conditions can be fully assessed.

Our results indicate that even in suitable sites, predation marks likely produced by the fireworm *Hermodice caraculata* and the corallivorous snail *Coralliophila gala* were present in 10–15% of *A. cervicornis* fragments surveyed. We also recorded loss of 14–26% of living tissues from January to October 2020, further suggesting this polychaetae hampers the survivorship of fragments. Similar results have been recorded in many restoration programs across the Caribbean (Hernández-Delgado et al., 2014). In addition to predation, macroalgae cover could be a significant factor influencing the success of coral outplants. In most cases, coral reef macroalgal cover near Bávaro has been reported > 40%. It was observed within our outplant sites, brown algae often smothered coral fragments, particularly those shorter than 120 mm in length. Thus, in areas where macroalgal cover is naturally high (60–70%), outplanting larger fragments (e.g., 150–250 mm) may be needed to increase survival success.

One of the biggest challenges to restoration success is the inadequate attention or control of threats causing reef decline and the need for restoration in the first place (Hein et al., 2020). Though we did consult with local experts on the influence of threats within our restoration site (i.e., temperature and history of coral bleaching, presence of predators/grazers, disease, turbidity/water quality, tourism activity, and land-based pollution), spatial data on these threats were not available and could not be incorporated into the modeling process. Thus, future research could more systematically take local anthropogenic and climate change-related threats into account in the site selection process relying on in-water surveys, expert guidance, and/or further modeling (Forsman et al., 2015).

[2]CaribbeanMarineMaps.tnc.org

Given the influence of local and climate threats on corals and the success of restoration efforts, more research into how threats can be incorporated into restoration site selection processes is warranted.

As suggested by Foo and Asner (2019), remote sensing, in particular hyperspectral sensing techniques, are currently underutilized and represent a key system in moving toward a more methodical way to identify reef restoration sites that have a heightened chance of surviving projected change. Remote sensing can provide information on abiotic conditions (e.g., water quality, sedimentation, and temperature) across much broader spatial and temporal scales when compared to diver surveys. When coupled with appropriate field surveys and spectral libraries, these technologies have the potential to detect between healthy and diseased corals, identify colonies at the species level, and are ideal approaches for monitoring restoration success. Additional research is needed to identify the ideal size, extent and position of an outplant area that will most likely facilitate the recovery of adjacent reefs and how outplanting influences reef connectivity and larval seeding (Foo and Asner, 2019). As remote sensing technologies are advancing rapidly, costs are becoming more affordable as new sensors and platforms evolve. When considering costs, remote sensing for restoration is more cost-effective if implemented through strategic partnerships between multiple agencies and organizations that are interested in collecting imagery over large areas which can greatly reduce the final cost of data collection and processing per hectare.

CONCLUSION

Advances in aerial mapping technologies have allowed mapping of broad reef ecosystems and the collection of multiple proxies of ecosystem health (e.g., coral and algal cover, rugosity, and structural complexity). Our research demonstrates for the first time the novel application of these tools to guide restoration efforts and locate sites that are suitable for outplanted corals. However, these technologies alone fail to quantify all of the important ecological, physical, and social processes and factors that are important for coral survivorship which require *in situ* assessments. A combination of broad-scale and fine-scale restoration technologies, conservation, and monitoring techniques represent the best approach. While we provide a proof-of-concept that broad-scale remote sensing is useful for selecting suitable sites for restoration, local restoration expertise is critical for identifying local threats, designing and implementing feasible conservation plans, and monitoring restoration success.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

GA collected the data. NV processed that data into data layers. SS and GR collected field data to validate the remote sensing products. SS, FP, AC, XE-F, and VM interpreted the data and worked with partners to select outplant sites. AC validated the data in the field, helped to define criteria for outplant sites, conducted monitoring and evaluation of the outplants, and analyzed monitoring data. VM developed the app. AC, VM, and SS generated the figures and tables. All authors designed the study, and drafted and edited the manuscript.

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Impact of Seawater Temperature on Coral Reefs in the Context of Climate Change. A Case Study of Cu Lao Cham – Hoi An Biosphere Reserve

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Coral reefs are a natural habitat for many species, as well as being of high economic and touristic significance. However, they represent an extremely sensitive ecosystem with a narrow ecological limit: prolonged high temperatures can lead to bleaching, in which corals expel their symbiotic algae and eventually corals will degrade and die. Based on climate change projections from the Blue Communities regional model, using linear regression, exponential regression, polynomial regression, we found that by the decades 2041–2050 and 2051–2060, whether with RCP 4.5 or RCP 8.5, the environmental temperature will change beyond the coral capacity threshold. Of particular concern is RCP 8.5, where the number of weeks per decade in which SST exceeds the threshold of coral reef bleaching is up to 55, compared to 0 at the beginning of the century. As well, the El Niño phenomenon often heats up waters to abnormally high temperatures in Cu Lao Cham and, it is projected to rise even further. Consequently, the combination of climate change and El Niño will cause abnormal increases in the seawater environment beyond the coral resistance threshold, leading to degradation of this internationally important site. Decisive and practical action must be taken to deal with climate change in this part of the world.

Keywords: biosphere reserve, climate change, El Niño, heat shock, coral reefs, Southeast Asian Seas

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INTRODUCTION

Coral reefs contain some of the highest levels of biodiversity among the oceans worldwide. Although they occupy less than 0.1% of the ocean floor, tropical coral reef ecosystems provide habitat for at least 25% of known marine species, with many reef species still to be discovered (Plaisance et al., 2011; Fisher et al., 2015), making it a key habitat for marine biodiversity. Furthermore, coral reefs only cover 0.1% of the seafloor because they grow in very specific environmental conditions: tropical seas with shallow warm water, but not overly warm; strong sunlight for the zooxanthellae algae's photosynthesis; and lack of turbidity (suspended particles in water tend to absorb radiant energy impeding the coral's filter-feeding ability, and can even bury corals, so corals are not usually found close to river mouth areas). At high nutrient levels, increased phytoplankton biomass reduces the clarity of the water, impeding the ability of the coral to filter-feed (Alan and Harold, 2011).

According to the Intergovernmental Panel on Climate Change (IPCC), climate change will lead to rising seawater temperature with serious adverse effects on coral reefs (Bindoff et al., 2019). High or largely fluctuating sea water temperatures can cause heat stress leading to expulsion of the zooxanthellae in an event known as coral bleaching; if the event is prolonged corals will degrade and die (Claar et al., 2018). The main cause of coral bleaching today is rising temperatures due to global warming. Bleached corals do not die immediately, but if the temperature is very hot, or too warm for a sustained period of time, they will die from hunger or disease. Warm-water coral reefs have declined by at least 50% over the past 30–50 years in large parts of the world's tropical regions (Gardner et al., 2003; Bruno and Selig, 2007). Under a moderate greenhouse gas emissions scenario (RCP 4.5) it is likely that most coral reefs will disappear during the period 2040–2050 (Hoegh-Guldberg et al., 2017).

Another risk factor for coral reefs is the occurrence of the El Niño phenomenon. El Niño is an extensive ocean warming event that begins along the coast of Peru and Ecuador and extends westward over the Tropical Pacific (Ahrens, 2009). It is the result of an abnormal interaction between the ocean surface layers and the atmosphere in the tropical Pacific. El Niño varies in a cyclical manner (Lyon, 2004) with an operation cycle lasting from 2 to 7 years, sometimes more than 10 years (Alan and Harold, 2011). The average duration of an El Niño event is 11 months, and the longest is 18 months (El Niño 1982 to 1983) (Lyon, 2004). Statistics show that El Niño has sea surface temperatures (SST) that are higher on average and with increased variability. Strong and very strong El Niño can change the biogeochemical environment, reduce coral cover and cause coral bleaching on a large scale (Hueerkamp et al., 2001; Podestá and Glynn, 2001; Claar et al., 2018; Hughes et al., 2018; Lough et al., 2018).

Previous work (Cai et al., 2014) showed that in the context of climate change, El Niño is becoming stronger. Thus, climate change combined with El Niño will make the temperature regime of seawater more disadvantageous to the coral ecosystem. This study looks at the potential effects of climate change and El Niño with a focus on one vulnerable coral reef system: Cu Lao Cham – Hoi An Biosphere Reserve in Vietnam. This region has rich biodiversity value (Nguyen, 2019), along with a strong cultural identity linked to the reef and harmony between nature and people. Therefore, this site has been recognised as a biosphere reserve under the UNESCO Man and the Biosphere program. The ecosystems of the Cu Lao Cham marine protected area (MPA), including coral reefs, have high natural value (Le, 2016).

Past research on Cu Lao Cham mainly used measurements of physical conditions, however, the collection of data was done at specific times, and was short-term and intermittent. To study the impacts of temperature on the coral ecosystem in Cu Lao Cham in the current context of climate change it is vital that longer-term data and projections of future conditions are attained. Vietnam is at risk of climate change and rising sea level scenarios. However, the parameters used for these scenarios include air temperature and rainfall, but only for the mainland (Tran et al., 2016). For the marine ecosystem, we will use future projection data from the GCRF Blue Communities project.[1] Thanks to this helpful source of data, research assessing the impacts of temperature on coral reef ecosystem development at Cu Lao Cham can for the first time be carried out without relying on scenarios developed for land areas.

METHODOLOGY AND DATA

The Study Area

Much research has been conducted on corals in Cu Lao Cham. A total of 277 species of hard coral reefs are estimated at 311.2 ha (Nguyen, 2017a). Coral reefs in Cu Lao Cham are narrowly distributed, mainly concentrated on the west and southwest coast of the big island and around most of the small islands (**Figure 1**). Almost all coral reefs in Cu Lao Cham are located in shallow water not exceeding 14 m (Nguyen, 2017b). One of the outstanding features in this area is that the ratio between hard and soft corals does not differ too much (hard corals account for 17.3–24.9%, soft corals account for 13.5–20.7%). Cu Lao Cham island and Hon Kho have the highest number of reef-creating hard coral species (79–80 species), followed by Vung Da Bao area (64 species), then Bai Bac, and Vung Da Den (53–57 species), while Bai Dau Tai area has the least species (15 species) (Nguyen, 2017b). The coral ecosystem in Cu Lao Cham is on the edge of serious decline due to tourism, excessive extraction of marine resources and climate change. Long-term research (Nguyen, 2017b) also showed that hard coral cover at most sites measured at Cu Lao Cham fell from 2004 to 2016. In the period 2004–2016, the coral area declined by 47%. The cover of live coral in Cu Lao Cham – Hoi An Biosphere Reserve is about 25% of the total biosphere reserve; within the reserve, coral in Vung Da Ban decreased by 91%; in Bai Bac by 78%, and in Bai Huong by 70% (**Figure 1**).

El Niño Events at Cu Lao Cham

From 1950 to 2021, there were five strong and three very strong El Niño (Jan Null and CCM, 2020). Corresponding to these El Niño warm events in the Pacific, Southeast Asian Seas (SEAS) also revealed a warm state (Wang et al., 2006) and the interannual SST anomalies over the SEAS show a double−peak feature following an El Niño event in the Pacific. The first and second peaks occur around February and August, respectively, in the year following the El Niño year (Wang et al., 2006). However, in February Cu Lao Cham SST is usually affected by the winter northeast monsoon which lowers the temperature so the first peak of the SST anomaly cannot cause heat shock. Subsequently, the risk of El Niño causing heat shock for SST in Cu Lao Cham will fall around August of the following year. This warming combined with the temperature anomalies in the context of climate change can cause heat shock for coral reefs.

Coral is strongly influenced by strong or very strong El Niño events (Ampou et al., 2017). Coral bleaching took place in the Pacific Ocean in 2016 (Brainard et al., 2018). The

[1] www.blue-communities.org

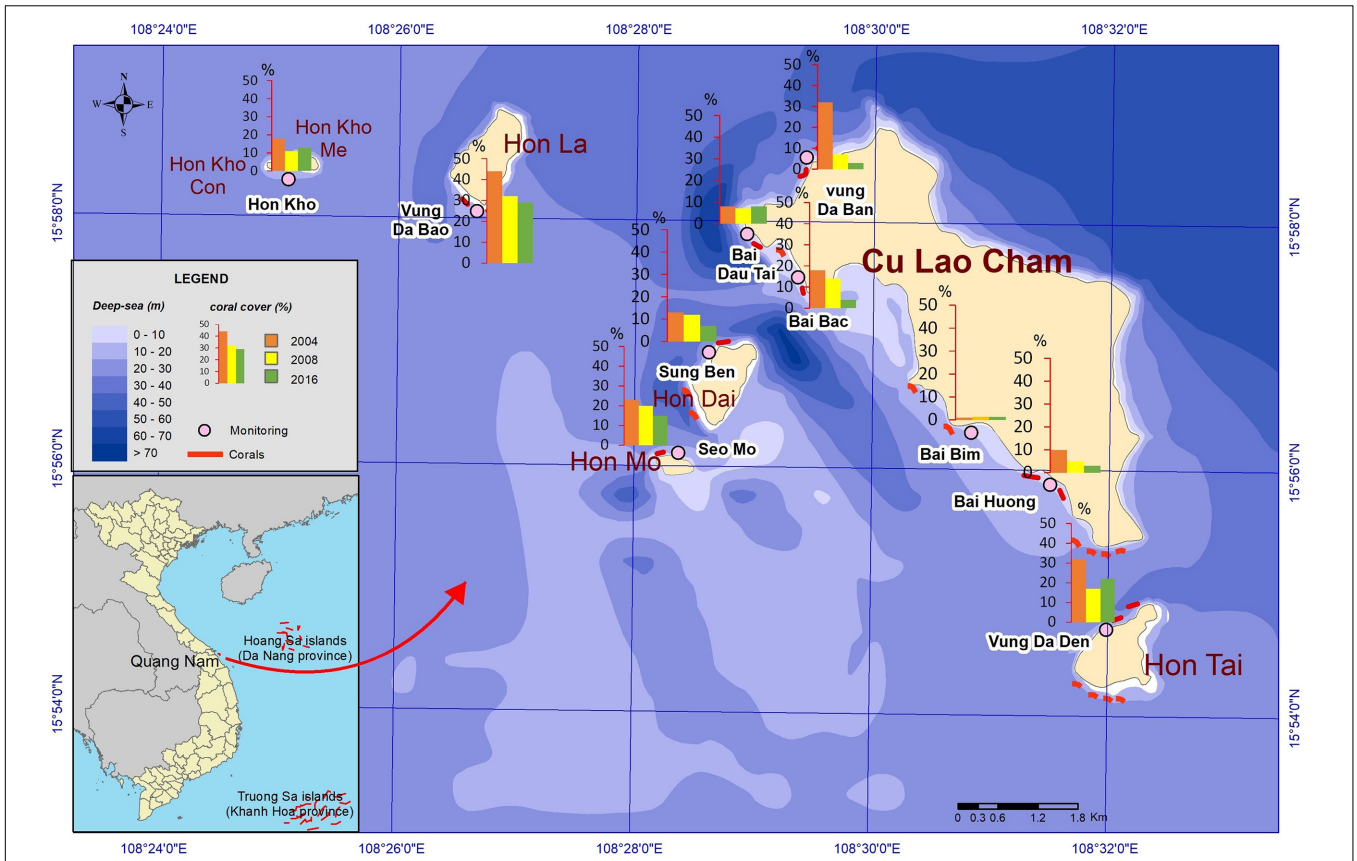

FIGURE 1 | The fluctuation of hard coral cover at monitoring sites by the time (Source data: Nguyen Van Long, 2017, pp. 155).

cause of this was the El Niño of 2015–2016, which had a strong event with the Oceanic Niño Index (ONI) reaching 2.1 (Climate Prediction Center, 2020). According to research for the Vietnam region (Pham, 2014), when El Niño occurred, the SST standard error of the SEAS (including Cu Lao Cham) increased compared to the surrounding area. As a result, seawater in Cu Lao Cham was hotter than usual. According to OISST (Optimum Interpolation Sea Surface Temperature) satellite monthly seawater temperature data, the average sea surface temperature in September 2015 was 30.25°C compared to an average September temperature of 29.3°C (Physical Sciences Laboratory, 2020).

Temperature Limit

Based on previous research (Kleypas, 1997; Guan et al., 2015) we determined ecological SST limits to the coral reef as follows:

Annual SST: 21.7–29.6°C (Guan et al., 2015),

Weekly SST: 18.1–31.5°C (Kleypas, 1997; Guan et al., 2015).

In this study, we calculated the average weekly temperature (from Monday to Sunday) during the historical period 1980–2005 and the period 2005–2060 under the scenarios RCP 4.5 and RCP 8.5 (see below). The results show the number of weeks in which the SST exceeded the threshold limit of corals reefs. SST is the highest in the June to August period in Cu Lao Cham. We found 5-year-average weekly SST in June to August period and compared these to the SST limit for coral reefs.

Projections of future temperature were created using the Proudman Oceanographic Laboratory Coastal Ocean Modelling System (POLCOMS) configured for SEAS (Holt et al., 2009). POLCOMS is a three-dimensional hydrodynamic model that simulates the physical processes of energy and momentum transfer in the ocean; its outputs include temperature, salinity, and current speed. It is suitable for use in a range of water depths from deep ocean to estuaries. The model resolution was 0.1° × 0.1° (about 11 km) and there were 40 vertical levels. Climate change was imposed by applying boundary conditions from a global climate model, HadGEM2-ES (Jones et al., 2011), taken from the Coupled Model Intercomparison Project Phase 5 (Taylor et al., 2012). Surface forcing (temperature, wind, pressure, humidity and radiation) were taken from a regional-scale atmospheric model, HadGEM2-ES-RCA4. Open ocean boundary conditions (temperature, salinity and currents) were taken from the global HadGEM2-ES. The model was run for the period 1970–2098, using initial conditions taken from the World Ocean Atlas 2018 (Locarnini et al., 2013). For 2006 onwards, two different Representative Concentration Pathways (RCPs) of greenhouse gases were applied by using different outputs of HadGEM2-ES, the moderate carbon RCP 4.5, which has greenhouse gas concentration rising until mid-century and

then stabilising, and the high carbon RCP 8.5, which has greenhouse gases continuing to rise throughout the 21st century (van Vuuren et al., 2011). The model was validated by comparing monthly mean SST for Cu Lao Cham, 1985–2018, to satellite-based values from the Operational Sea Surface Temperature and Ice Analysis product (OSTIA) (Good et al., 2020) downloaded from the Copernicus Marine Service.[2]

Model outputs for the period 1980–2060 were analysed to confirm the trend in temperature rise. Model outputs analysis also explained the number of weeks per decade calculation under each scenario, in an effort to find the number of weeks where the temperature exceeds the coral reefs' threshold limit (31.5°).

Correlation Coefficient

The correlation coefficient between two random quantities X and Y is the mathematical expectation of the product of their standardised deviations (Taylor, 1997).

$$R^2 = \frac{\left(\overline{X * Y} - \bar{X} * \bar{Y}\right)^2}{\left(\overline{X^2} - \left(\bar{X}\right)^2\right) * \left(\overline{Y^2} - \left(\bar{Y}\right)^2\right)}$$

To construct a regression equation, $R^2 > 0.5$ is acceptable.

Regression

The straight line that best matches the correlation between x and the conditional mean $\hat{y}$ is called the linear regression (Taylor, 1997).

$\hat{y} = ax + b$ (regression)

where $a = \frac{\overline{X*Y} - \overline{X} * \overline{Y}}{\overline{X^2} - \left(\overline{X}\right)^2}$; $b = \overline{Y} - a\overline{X}$

$\hat{y}$ is the outcome criterion calculated from the regression equation,

a is the slope, reflecting the reflection $\hat{y}$ depending on x,

b is a free coefficient, the reflection independent on x.

In this study, an exponential regression is used to describe the relationship between SST and time; polynomial regression is applied to express the relationship between ONI and time.

RESULTS

Model Validation

To assess how well the model outputs match observed sea surface temperatures, monthly mean model values from POLCOMS and from its parent global model, HadGEM2-ES, were plotted against satellite-based observations (**Figure 2**). Values for both RCP4.5 and RCP8.5 were plotted for 2006 onwards, but for this period there is no systematic difference between the two scenarios and they can be treated as two different runs of the model. The POLCOMS outputs show good agreement with the range and variability of observed temperature. At the lower end of the temperature range the model tends to overestimate the temperature, but for higher temperatures, which are the concern of this study, the bias is close to zero. HadGEM2-ES overestimates temperatures at all parts of the range and shows greater variability than the observations: this illustrates the value of using a regional model for this coastal site.

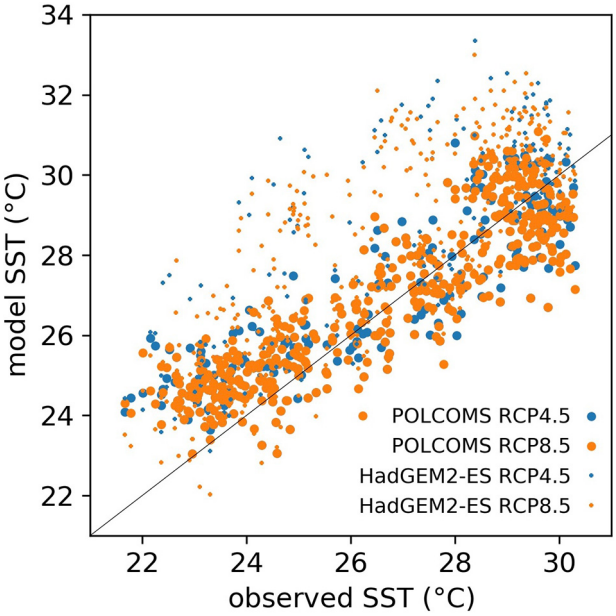

FIGURE 2 | Scatter plot between observed SST and model SST.

Annual Fluctuation of Sea Water Temperature in the Cu Lao Cham Biosphere Reserve

Using a climatology model based on the years 1980–2005, we found that the monthly average SST reaches its highest value in June at 29.6°C and is lowest in January at 24.4°C (**Figure 3**). Thus, the temperature here has seasonal fluctuations, with an annual amplitude of temperature fluctuations of 5.3°C. In the years 1980–2005 the annual average SST maximum was 27.2°C and the minimum was 26.1°C (**Figure 4**). These values are well within the ecological limits of coral reefs (21.7–29.6°C).

Assessment of Changes in SST According to the Historical Scenario

The yearly fluctuation and changing trend of SST according to the historical, RCP 4.5 and RCP 8.5 scenarios are shown in **Figure 4**.

According to the RCP 4.5 scenario, the linear fit for temperature rise has a coefficient of determination $R^2 = 0.5$, indicating a reliable trend of annual increase in temperature. The results obtained from the regression equation show that the average annual seawater surface temperature increases by 1.63°C for 100 years. Under RCP 8.5, it can be seen from **Figure 4** that there is a close correlation between the rise in temperature and time with $R^2 = 0.73$, meaning that the annual increase trend in temperature is reliable. It can be observed from the regression equation that the annual seawater surface temperature will increase by 2.98°C over 100 years on average.

[2]marine.copernicus.eu

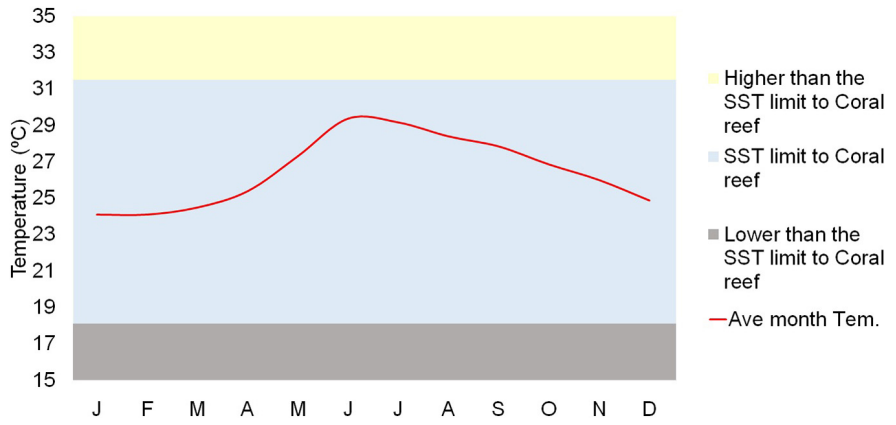

FIGURE 3 | Monthly averages of sea surface temperature in Cu Lao Cham sea (16.0°N–108.5°E) in period 1980–2005.

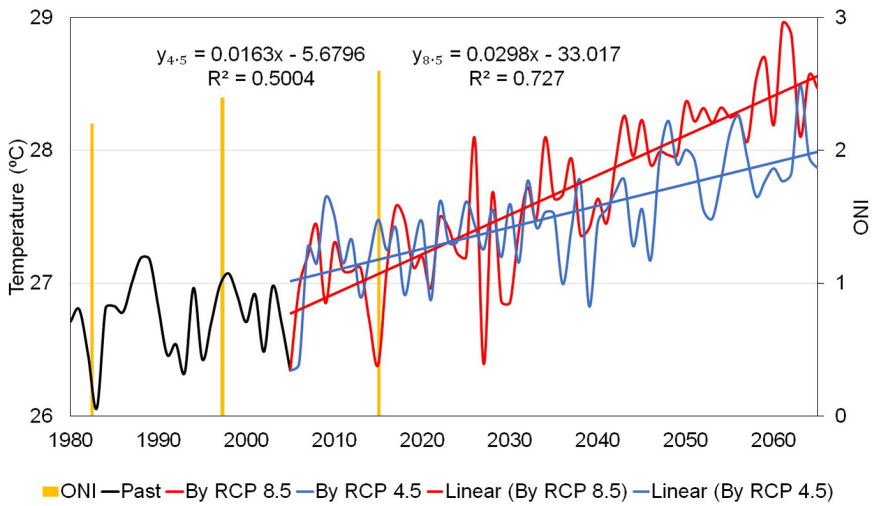

FIGURE 4 | Modelled historic and future annual average sea surface temperature, showing a linear fit to the trend for 2005–2060 under RCP 4.5 (blue) and RCP 8.5 (red). The Oceanic Nino Index for years with strong and powerful El Nino is shown by vertical.

In conclusion, in the context of climate change it is estimated that annual SST will increase between now and 2060. Annual SST increase according to RCP 8.5 is higher than for RCP 4.5 is 0.0135°C/year.

Number of Weeks per Decade Having an Average SST Exceeding the Limits for Coral Reef Development

Figure 5 shows the number of weeks per decade where the average temperature is above the upper temperature limit of coral (31.5°C). According to the RCP 4.5 scenario, in the decades 1981–1990, 1991–2000, 2001–2020, and 2011–2020 the coral resistance threshold was not exceeded. However, the next decade (2021–2030) contained a week having temperature exceeding the coral resistance threshold. In the decades 2041–2050 and 2051–2060, there were 22 and 10 weeks, respectively, where the temperature exceeded the upper limit of coral.

According to the RCP 8.5 scenario, the number of weeks having temperatures above 31.5°C significantly increased over time. Especially in the coming decades (2031–2040, 2041–2050, and 2051–2060) the number of weeks having an average temperature exceeding the threshold increased significantly. It is worth noting here that the number of weeks having a temperature exceeding the threshold increases by the function $y = 0.74e^{0.87x}$ with a very high reliability level ($R^2 = 0.9745$). In the decade 2051–2060, the number of weeks exceeding the threshold was highest, at 55.

Thus, weekly SST exceeding 31.5°C, the capacity of coral reefs, has never happened in the past but will occur regularly in the future. According to the RCP 8.5 scenario, SST in 2051–2060 will have 55 weeks exceeding the threshold, twice that of the RCP 4.5 scenario in 2041–2050. The number of weeks in which SST exceeds the temperature limitation of coral reef increases exponentially according to the RCP 8.5 scenario.

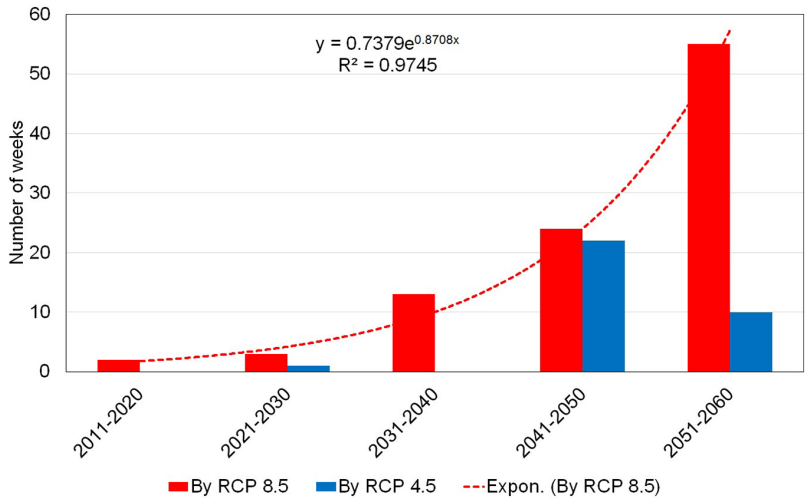

FIGURE 5 | Number of weeks having a temperature exceeding the ecological threshold of coral-31.5°C.

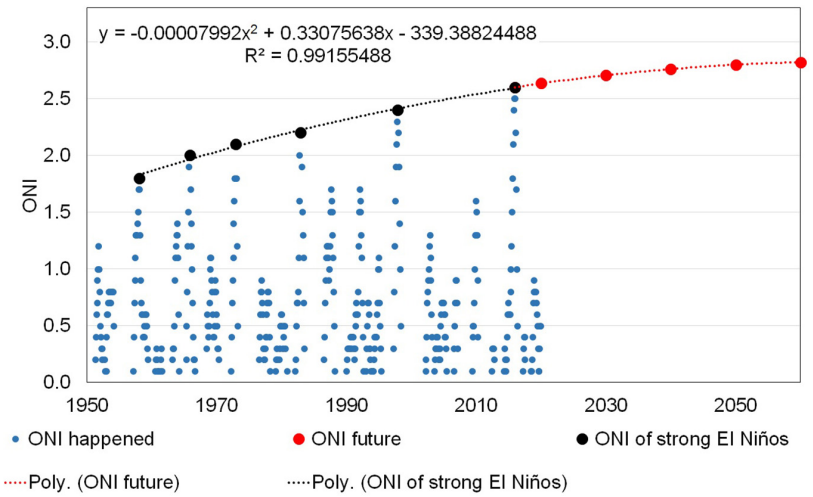

FIGURE 6 | Oceanic Nino Index values for 1958–2020 (blue clots). Strong and powerful ElNmos (Oceanic Nino Index > 1.5) are shown with red dots. The line shows the observed trend of strong and powerful events; this is extrapolated to illustrate possible future.

El Niño and the Warming Seawater

ONI data for 1950–2010 (Climate Prediction Center, 2020) served to identify years with strong and very strong El Niño episodes (>1.5 ONI Index value). It can be seen from 50 years of records concerning strong and very strong El Niño, a trend for strong and more very strong El Niño is evident, with average ONI increasing by years according to the equation:

$$y = -0.0000799x^2 + 0.33075x - 339.3882 \quad (1)$$

where x is the year; y is ONI.

This equation was used to estimate future values for the ONI in strong El Niño years to 2060 (**Figure 6** red dots). In the future, based on eq. 2, if very strong El Niños occur, the ONI value will increase as the years pass. Estimated ONI values for very strong El Niños in future years (**Figure 6**): by 2030, if very strong El Niños occur, the ONI value could reach 2.7; by 2050–2060 it could reach 2.8.

So, it can be judged that in the future, the intensity of El Niño will be much stronger than what is currently happening, and the subsequent sea surface temperature anomalies will be higher. Their impact on the coral reefs will be more serious than ever before.

Increase in SST Due to the Combination of Climate Change and El Niño

As mentioned previously, the risk of El Nino causing heat shock for SST in Cu Lao Cham will fall around August. **Figure 7** shows the 5 years averages of the weekly mean temperature and how these compare to the coral temperature tolerance window. As previously note the hottest months are June and July, closely

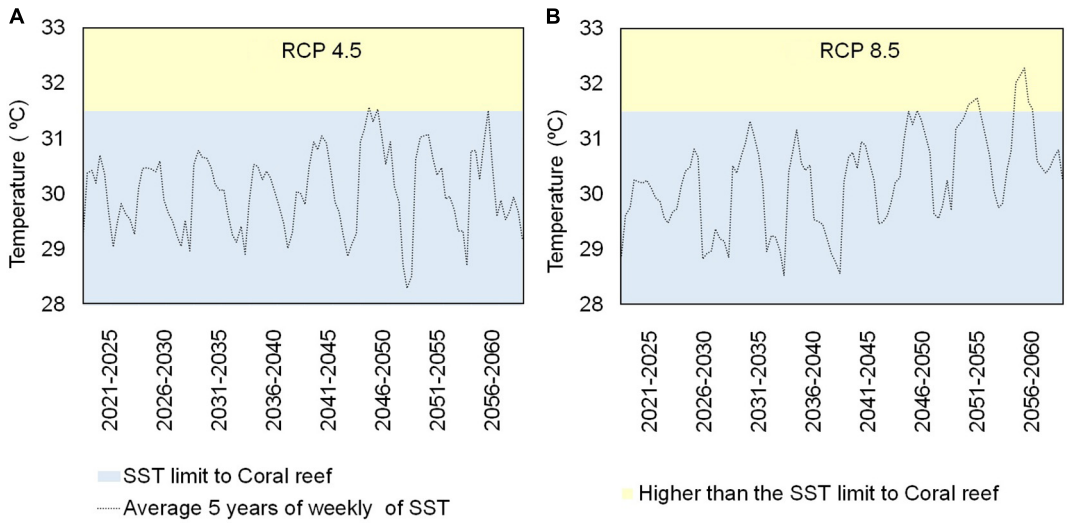

FIGURE 7 | Variation of 5 years average of weekly sea surface temperature in June, July, and August for the period 2021–2065 in Cu Lao Cham. The blue region shows the temperature range in which coral are able to survive.

followed by August. Under RCP 4.5, the number of weeks with an average SST that exceeds the upper limit of coral reef in 2020–2060 increases by 36% in June; 58% in July, and 6% in August. In the other months, there is no week that SST is higher than the threshold capacity of the coral reefs. Similar under RCP 8.5, the number of weeks with an average SST exceeding coral reef upper limit in 2020 to 2060 increases by 42% in June, 47% in July, 10% in August, and 1% in September. There are no other months that are projected to witness an increase in SST above corals' upper limit under RCP 8.5. Consequently, the combination of high weekly average SST with El Niño is most likely to happen in July and August.

DISCUSSION

The SEAS is a warm sea meaning that the average sea water temperature is higher than other ocean in the world (Physical Sciences Laboratory, 2021). Results of this is the number of storms is also higher compared with other places (Gray, 1968; Matsuura et al., 2003; Nguyen and Nguyen, 2004). Located in the SEAS, Cu Lao Cham experienced high SST. Therefore, SST is one of the most important factors in assessing the impact of climate change on coral in this area.

Monitoring data about coral in this area are very limited. Coral cover data is only available for 2004, 2008, and 2016, which is not sufficient for a correlation analysis. In addition, the decline of coral in this period may be caused by factors other than temperature change (tourism and excessive extraction of marine resources), and there is no method of how to extrapolate these factors into the future. Therefore, this study is based on results modelling from previous researches.

Impact of seawater temperature on coral reefs in this paper are projections of what is likely to occur which is based on the following assumptions.

Reliability of Ecological Limit

The ecological limit is 31.5°C inherited from research of Suitable environmental ranges for potential coral reef habitats in the tropical ocean (Guan et al., 2015). This limit is widely used in many other studies. For example, the Warming of Coral Reefs in the Florida Keys (Manzello, 2015), Research about coral reefs in Taiwan's sea (belong to SEAS) (Mayfield et al., 2013).

Reliability of Model

To assess how well the model outputs match observed sea surface temperatures, monthly mean model values from POLCOMS and from its parent global model, HadGEM2-ES, were plotted against satellite-based observations (**Figure 2**). Values for both RCP4.5 and RCP8.5 were plotted for 2006–2018, but for this period there is no systematic difference between the two scenarios and they can be treated as two different runs of the model. The POLCOMS outputs show good agreement with the range and variability of observed temperature. At the lower end of the temperature range the model tends to overestimate the temperature, but for the higher temperatures which are the concern of this study the bias is close to zero. HadGEM2-ES overestimates temperatures at all parts of the range and shows greater variability than the observations: this illustrates the value of using a regional model for this coastal site.

Assess the Impact of El Niño

It is claimed that strong EN Nino have reduced coral cover. This study mainly inherits conclusion from other studies. Statistical results show the intensity trend of El Niño events is expected to increase. Research by Wang et al. (2006) also show that the consequences of El Niño are positive temperature anomalies in the SEAS. According to NOAA Optimum Interpolation (OI) finds that the SST in the SEAS and Cu Lao Cham sea during the El Nino periods is higher than in

other areas. In conclusion, El Niño will increase the temperature in Cu Lao Cham sea.

Although exposure to high water temperatures can be fatal to coral growth, this study is unique in that it assessed the future effects of two thermal stresses: global warming and El Niño.

CONCLUSION

Climate change has happened throughout the history of the Earth. The evidence found in isotope signatures in fossils shows that Scleractin corals were symbiotic with Symbiodinium for more than 240 million years, most of which could control diverse ecosystems, and functioning in ways that are not too different from today (Muscatine et al., 2005). So, it can be based on the ecological limits given by Kleypas and Guan (Kleypas, 1997; Guan et al., 2015) for assessing the environmental impact on the coral reef for the present and the future.

Our findings highlight that under either RCP scenarios there is a risk of experiencing a decline in coral reefs in Cu Lao Cham, which is especially true for the 2040 and 2050 decades. Beyond the overall increase in temperature there is an increase in how often these temperatures will exceed the temperature preference of coral. This is more pronounced under RCP 8.5, with up to 55 weeks per decade where the temperature is projected to exceed the coral threshold. In addition to the rise in temperature, El Niño strength is projected to increase in the future, and we found that the effect of El Niño is generally impacting the area in August, extending the period of potentially too high temperature from June-July (the two hottest months of the year in the historical period) by one month. The combination of the increase in temperature potentially followed by an El Niño – a stronger and longer El Niño event than at present – would result in a prolonged period of extreme temperature from which the coral reefs of Cu Lao Cham will very likely not recover.

The findings reported here are clear, but they are based on projections made using a single global climate model, downscaled using a single regional model. This initial work needs to be repeated using a range of climate models to: firstly, give greater confidence in the findings; and secondly, make it possible to estimate the uncertainty in the risk of future high-temperature events. Given the coastal location of Cu Lao Cham, regional-scale projections are more appropriate than global models and more regional marine modelling studies are needed to expand on this work. Our research on El Niño was based on extrapolation from multi-decade observations; as the representation of El Niño in climate models improves it will be become possible to base this aspect of the work on models as well.

The combination of weeks having heat shock in the future and El Niño means that the survival of coral reefs in Cu Lao Cham is indeed at great risk. Hopefully, the findings of this research will have practical implications, helping environmental management agencies by providing valid sources of data what will greatly help the biosphere reserve's conservation.

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.

AUTHOR CONTRIBUTIONS

HD: conceptualisation, methodology, and writing – original draft. HD: visualisation. SK: writing – review and editing. SS: writing – review and editing and data curation. All authors contributed to the article and approved the submitted version.

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Should Hybrids Be Used in Coral Nurseries? A Case Study Comparing Caribbean *Acropora* spp. and Their Hybrid in the Bahamas

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For decades, coral reef ecosystems have been in decline due to environmental stressors such as rising sea temperatures, increased disease prevalence, and other local anthropogenic sources. Considering this decline, coral restoration efforts in the Caribbean have been implemented to promote reef recovery with a focus on the coral genus *Acropora.* Current methods target the threatened species *Acropora cervicornis* and *A. palmata*, but little is known about the restoration potential of their hybrid taxon, *A. prolifera*. Using interspecific hybrids with higher fitness than one or both parental species has gained traction as a novel restoration technique. For this study, three *in situ* coral tree nurseries were established around Great Stirrup Cay, The Bahamas, to compare the growth and survival among acroporid taxa. Three 150 mm fragments from six putative genotypes of each acroporid taxa were collected from reefs around New Providence, The Bahamas, and transported to Great Stirrup Cay in June 2018. One fragment from each genotype was transported to each nursery site, cut into three sections (apical, middle, and basal), and suspended from PVC coral trees. Fragment survival was collected monthly for 13 months, and Total Linear Extension (TLE) values were calculated for each fragment monthly for 12 months. Nursery site significantly affected fragment survival, while taxon and fragment section did not. Total fragment mortality was 29.3% in the first month but ranged from 0 to 5% for the rest of the study period until July 2019 (32.7% of remaining fragments died primarily at N1). Overall, *A. prolifera* growth was significantly greater than the parental species. Taxon, nursery site, and fragment section were identified as important factors affecting TLE. Apical *A. prolifera* fragment sections at site N3 had the greatest average linear growth at 12 months and had the greatest average growth rate per month. This study highlights the rapid growth rate of hybrid corals and suggests that fragment sections have equivalent survival and growth. Consequently, these results suggest that restoration managers may capitalize on fast growing hybrids for outplanting to degraded reefs and to increase the scale of nursery projects.

Keywords: *Acropora*, hybrid, coral restoration, coral nursery, Caribbean

INTRODUCTION

In the face of climate change and other environmental threats, conservation and restoration of the world's natural resources are now at the forefront of scientific research (Harris et al., 2006; Heller and Zavaleta, 2009; Jackson and Hobbs, 2009). Global issues such as deforestation, rising global temperatures, pollution, and the overuse of natural resources are serious threats to marine ecosystems and terrestrial environments (Vitousek, 1994; Derraik, 2002; Shahidul Islam and Tanaka, 2004; Harley et al., 2006; Malhi et al., 2008; Cinner et al., 2015; Hughes et al., 2017b). As such, finding ways of protecting these environments are critical to the continuity of the global biome.

Coral reefs are one of the world's most important and threatened marine ecosystems (Sebens, 1994; Maragos et al., 1996; Reaka-Kudla, 1997; Hughes et al., 2017a). They host a diversity of ecologically and commercially important marine species (Moberg and Folke, 1999), are essential nursery grounds for numerous fish and invertebrate species (Heck et al., 2008; Holbrook et al., 2015), protect coastlines from storm damage (Cesar et al., 2003; Ferrario et al., 2014; Storlazzi et al., 2019), and support an extensive tourism industry for many island nations and coastal regions (Cesar et al., 2003). Unfortunately, nearly 27% of the world's coral reefs have been lost due to destructive events and stressors (Cesar et al., 2003). Local anthropogenic threats (e.g., physical damage, overfishing, pollution, sedimentation), and larger global stressors (e.g., temperature increases, increased disease prevalence and storm damage, ocean acidification), are drivers of coral decline (Lirman and Fong, 1997; Hughes and Connell, 1999; Babcock and Smith, 2000; Woodley et al., 2000; Bellwood et al., 2004; Shahidul Islam and Tanaka, 2004; Fox and Caldwell, 2006; Voss and Richardson, 2006; Pandolfi et al., 2011; Smith et al., 2015; Cheal et al., 2017; Hughes et al., 2017b; Hughes et al., 2018). Increased sea temperatures can cause coral bleaching, a stress response during which colony pigmentation may be lost and often the microalgae symbionts found in coral tissue are expelled (Brown, 1997; Baker et al., 2008; Heron et al., 2016), which can lead to colony death if the stress is prolonged (Douglas, 2003; Eakin et al., 2010). Increasing ocean temperatures have also been linked to increases in disease outbreaks, resulting in large-scale coral mortality (Weil, 2004; Harvell et al., 2007; Muller et al., 2007; Eakin et al., 2010). Disturbance to a reef is natural but continued impacts of chronic stressors has changed coral reef community composition from one benthic group to another (Hughes et al., 1985; Hughes, 1994; Lirman, 2001; Bellwood et al., 2004; Knowlton and Jackson, 2008; Jackson et al., 2014; Jones et al., 2020).

In the Caribbean, the increase of such stressors and their compounding effects has led to significant losses in scleractinian coral cover since the late 1970s (Gardner et al., 2003; Eakin et al., 2010; Jackson et al., 2014). Much of this loss is attributed to the severe decline (up to 95%) of the Caribbean acroporid corals, *Acropora cervicornis* and *A. palmata* (Aronson and Precht, 2001; Bruckner, 2002). Prior to the 1970s, *A. cervicornis* and *A. palmata* were the major contributors to many reef habitats across the Caribbean (Aronson and Precht, 1997; McNeill et al., 1997; Bruckner, 2002; Gardner et al., 2003; Miller and van Oppen, 2003; Bellwood et al., 2004), contributing up to 50% of total stony coral cover above ∼20 m depth (Bellwood et al., 2004). These species provide many ecosystem services, including vital habitats for fish and invertebrates, reef structure through carbonate deposition, and coastal wave protection from storms (Bruckner, 2002). Acroporids primarily rely on asexual reproduction through fragmentation (Rinkevich, 1995; Lirman and Fong, 1997; Smith and Hughes, 1999), but also reproduce sexually through hermaphroditic broadcast spawning (Szmant, 1986; Vargas-Angel and Thomas, 2002; Fogarty et al., 2012). The two parental species are also capable of reproducing with each other to produce an F1 hybrid, *Acropora prolifera* (van Oppen et al., 2000; Vollmer and Palumbi, 2002; Kitchen et al., 2019). Like the parental species, *A. prolifera* can reproduce asexually through fragmentation, and the molecular signatures suggest they reproduce sexually with the parental species (Vollmer and Palumbi, 2002; Kitchen et al., 2019; Kitchen et al., 2021). In recent decades, *A. cervicornis* and *A. palmata* have declined in abundance primarily from disease, but also bleaching, storm damage, and predation (Aronson and Precht, 2001; Bruckner, 2002; Jackson et al., 2014). In response to their decline, *A. cervicornis* and *A. palmata* were listed as threatened under the United States Endangered Species Act as of 2006 (National Marine Fisheries Service, 2006) and as critically endangered by the International Union for the Conservation of Nature (IUCN)'s Red List as of 2008 (Aronson et al., 2008a,b). In recent years, hybrid abundance has increased at some sites in the Caribbean, despite losses in the parental species (Fogarty, 2010, 2012; Japaud et al., 2014; Nylander-Asplin et al., 2021).

To facilitate recovery of *A. cervicornis* and *A. palmata,* many organizations through the Caribbean are working to increase *Acropora* abundance and genetic diversity (Johnson et al., 2011; Young et al., 2012; Baums et al., 2019; Boström-Einarsson et al., 2020). In many cases, these efforts are achieved by the creation and maintenance of coral nurseries, which provides a sheltered area for corals to grow away from the reef and predators. This 'gardening technique' proposed by Rinkevich (1995) has been adopted as a general practice for many reef restoration organizations. Based on silviculture practices, coral fragments are collected from different genotypes of the target species, grown in *in situ* nurseries, and outplanted to local reefs (Rinkevich, 1995; Lirman et al., 2010; Lirman, 2000; Zimmer, 2006). This method of coral gardening has been widely adapted across the globe for large-scale restoration efforts.

Caribbean *Acropora* restoration research has led to improvements in propagation techniques, as well as the recovery of localized populations of the parental species (Ware et al., 2020), but there is limited information and use of the hybrid in restoration. While the parental species have been in decline, the hybrid has persisted on many reefs in the Caribbean with equal or increased abundance, better survival, and equal or less susceptibility to disease and other environmental pressures (Fogarty, 2012; Irwin et al., 2017; Howe, 2018; Nylander-Asplin et al., 2021; Weil et al., 2020). Furthermore, research on the early life stages of *Acropora* species in the Pacific suggests hybrid larvae had equal or greater fitness compared to the parental species (Chan et al., 2018, 2019b). Although hybrids are often

thought to be sterile (Ortiz-Barrientos et al., 2007), *A. prolifera* can successfully reproduce with both *A. cervicornis* (Kitchen et al., 2019) and *A. palmata* via backcrossing (van Oppen et al., 2000; Vollmer and Palumbi, 2002; Kitchen et al., 2019). Backcrossing is noted among many marine organisms (Arnold and Fogarty, 2009) and provides an avenue for the genetic material from one parent to be exchanged between congenerics, that may have led to reticulate evolution (Veron, 1995; Willis et al., 2006). Backcrossing may enhance the adaptive potential of the threatened parental species in a changing environment by providing increased genetic diversity (Willis et al., 2006), or at the very least, acroporid hybrids may provide needed infrastructure to shallow reefs while the parental species continue to decline.

To identify the potential of using hybrids in restoration, this study investigates factors (nursery site, taxa, fragment section, and genotype) that may influence growth and survival of the threatened Caribbean acroporid coral species and their naturally occurring hybrid at three *in situ* nurseries in The Bahamas.

MATERIALS AND METHODS

Study Location

This study was conducted at Great Stirrup Cay (GSC) (25.824 N, −77.91 W), The Bahamas, from June 2018 to July 2019. Great Stirrup Cay is located at the northern end of the Berry Islands in the central Bahamas (**Figure 1**). Great Stirrup Cay is a private island owned by Norwegian Cruise Line® (NCL), which receives thousands of cruise ship visitors every week. Coral reefs fringe the northern side of the island, and seagrass beds and sand flats are common to the south. The deeper fringing reefs (∼15 m) on the northern side of the island are composed of large mounding corals including *Orbicella* spp. and *Montastraea cavernosa*, gorgonians, and sponges. On the eastern side of the island, reefs flats contain scattered acroporid colonies and smaller mounding corals, along with various species of gorgonians.

Study Species

Acropora cervicornis is typically found on shallow reefs down to 20 m depths; *A. palmata* is usually found on shallow reef crests to 10 m depths in areas with high wave energy (McNeill et al., 1997; Johnson et al., 2011). *Acropora palmata* can grow up to ∼10 cm per year (McNeill et al., 1997; Bak et al., 2009), while *A. cervicornis* has been recorded growing much faster, depending on location and genotype (Gladfelter et al., 1978; Lirman et al., 2014; Schopmeyer et al., 2017). *Acropora cervicornis* has long, thin branches extending from a central basal attachment, while *A. palmata* has wide, flattened branches that also extend from a central basal attachment point (Neigel and Avise, 1983). *Acropora prolifera* is described as having a "bushy" or "palmate" morphology that is intermediate between the parental species and originally attributed, at least in part, to the maternal species (Vollmer and Palumbi, 2002). Recent molecular evidence from hybrid samples across a broader geographic range suggests egg donor is not predictive of hybrid morphology (Kitchen et al., 2021). Hybrids are often found at shallow depths (<2 m) with moderate to high wave energy, but occasionally can be found in deeper, calmer environments (Fogarty, 2012). All three taxa can naturally reproduce asexually via fragmentation, making them ideal candidates for coral restoration (Rinkevich, 1995; Herlan and Lirman, 2008; Griffin et al., 2012; Schopmeyer et al., 2017).

Nursery Sites

Three replicate nursery sites were established at GSC. Nursery locations included two southern sand flat sites (N1 and N2) and one northern reef slope site (N3) with depths ranging from 2.5 to 3.5 m (**Figure 1**). Site N1 was located near adjacent seagrass beds, while site N2 was established in sand near a boat channel on the western side of the island. Site N3 was the most exposed nursery site in terms of wave action and seasonal wind patterns. Sites were chosen based on depth, accessibility, storm protection, and isolation from human impact. Nursery sites were not placed on the east end of the island near wild *A. cervicornis* and *A. palmata* colonies due to limited accessibility from high wave energy and shallow depths. Turbidity was dependent of time of year, with site N1 observationally having greater turbidity than sites N2 and N3 but better protection from wave action due to seasonal weather patterns and storms. Three coral nursery trees© (Nedimyer et al., 2011) were placed 5 m apart in a line at each nursery site. Nursery trees were made from PVC and fiberglass rods with pre-drilled holes along each rod. The trees were tagged and secured to the seabed using sand (helix) anchors or epoxied eyebolts, depending on the substrate type (sand or hard bottom, respectively). Trees were tied to the anchors using polypropylene rope with plastic tubing through a metal shackle, such that the middle branch was at a depth of approximately three meters below the surface. Every tree contained five branches with corals attached to the middle three branches. Each branch was spaced 15 cm apart and installed perpendicular to the one above to avoid abrasion and shading effects between coral fragments. Six corals were attached per branch approximately 10 cm apart using 80 lb. test (0.89 mm) monofilament.

Coral Collection

Acropora cervicornis, *A. palmata*, and *A. prolifera* fragments were collected from the reefs around New Providence, The Bahamas, in June 2018 using a hammer and chisel or diagonal cutters. Fragments were collected from colonies between 1 and 3 m depth and ≥10 m apart to increase the confidence of genotypic variation. Collection targeted six putative genotypes for each taxon; a small tissue sample was collected to confirm clonal identity. Three 150 mm branches (fragments) were collected from each donor colony (n = 19 *A. cervicornis*, n = 18 *A. palmata*, n = 18 *A. prolifera*). An extra branch from a separate colony was collected for *A. cervicornis* due to greater availability and the potential loss of other fragments due to stress of transport. *Acropora palmata* and *A. prolifera* were not as widely available, and so only the minimum number of branches were collected. The three branches from each colony were placed in heavy duty plastic zipper bags filled with seawater and transported inside of Bubble Wrap® lined coolers. Ice packs were placed in the coolers for temperature regulation and were flown to GSC. Upon arrival the water was changed and the fragments were transported to nursery site N2. The following day, one 150 mm fragment

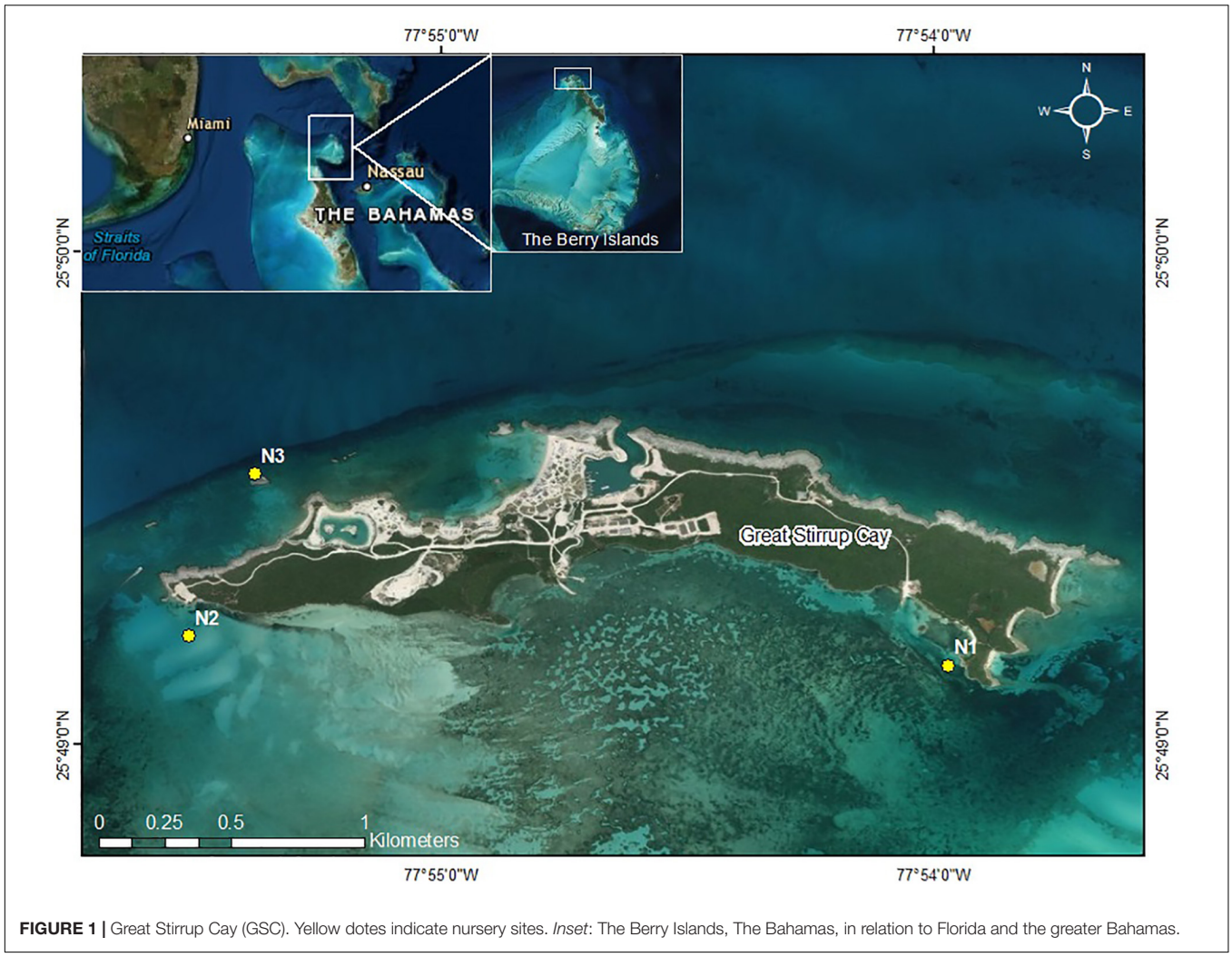

FIGURE 1 | Great Stirrup Cay (GSC). Yellow dotes indicate nursery sites. *Inset*: The Berry Islands, The Bahamas, in relation to Florida and the greater Bahamas.

from each genotype was transported to each nursery site and cut into three smaller 50 mm sections (n = 57 *A. cervicornis*, n = 51 *A. palmata*, and n = 49 *A. prolifera*). The sections were labeled as apical, middle, and basal, as per origin on the donor branch (**Figure 2**) and were distributed across the three trees at each site (**Figure 3**). Five *A. prolifera* and three *A. palmata* fragments were not included in the nursery due to poor visual condition after transport, and three extra *A. cervicornis* sections were included from initial collection. Note, this created a slightly uneven sampling design. All taxa, putative genotypes, and fragment sections were replicated at each site in a crossed design (see **Supplementary Figure 2**). However, later genetic analysis revealed some of the putative genotypes were clone mates and therefore contained more fragments. Tree location, coral section size (length, width, and size/number of branches), and condition data were recorded immediately. Each section was marked by a metal tag attached to the branch of the trees above each coral. An Onset HOBO pendant temperature/light logger was attached to one tree at each site and recorded temperature data every 2 h to capture daily fluctuations and maximize the length of deployment based on available memory.

Data Collection

Nursery sites were visited monthly between June 2018 and July 2019, during which the trees were cleaned and data were collected. Data included total length, total width, number of branches >10 mm, branch length(s), % mortality, and condition data (presence/absence of disease, predation, bleaching). Size measurements were taken with calipers to the mm. Images were also taken of each fragment with a scale bar. Linear extension was measured in ImageJ if branch measurements could not be completed in the field (ImageJ Version 1.52n, 2018).

Genetic Analysis

Genetic samples were collected from all donor colonies. A small ∼10 mm sample was cut and placed in a ∼1 mL centrifuge tube and filled with 96% molecular grade ethanol. DNA was extracted using magnetic bead protocol, as described in Fogarty et al., 2012. This was followed by PCR amplification using five microsatellite markers (Fogarty et al., 2012 modified from Baums et al., 2005). After fragment analysis (conducted at Florida State University), peaks for each fragment loci were analyzed using

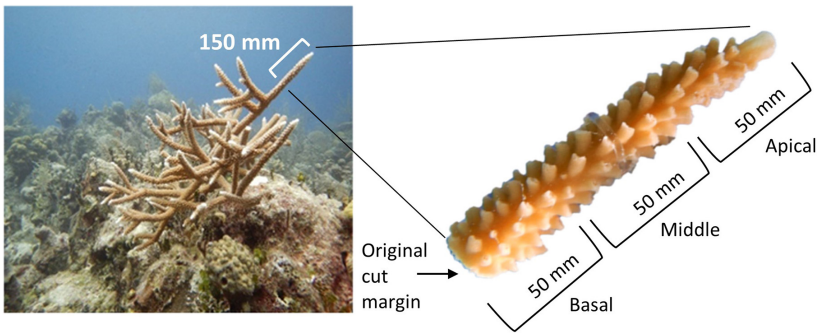

FIGURE 2 | *Left*: *Acropora cervicornis* colony showing collection fragment (~150 mm). *Right*: Sections of larger 150 mm fragment. Fragment section was designated from the portion of the donor fragment: the first 50 mm (proximal end) were considered the apical fragment section, the next 50 mm were the middle fragment sections, and the interior most 50 mm of the donor fragment were the basal fragment sections.

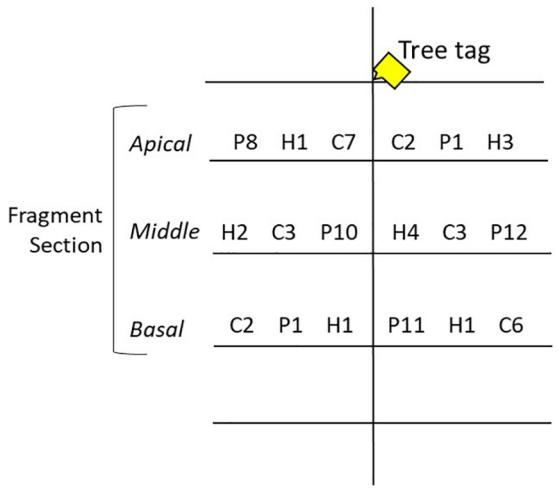

FIGURE 3 | Nursery tree experimental setup. Coral fragments were placed on the middle three tree branches shown here with a letter and number value. Fragment sections were individually tagged. C, P, and H denote taxa [*A. cervicornis, A. palmata,* and *A. prolifera* (hybrid), respectively], and numbers denote genotype. The fragment section changed branches between trees at each site. See **Supplementary Figure 2** for full experimental design.

GeneMapper 5^{TM} software. Unique and clonal genotypes were identified using the Excel microsatellite toolkit (Park, 2001). To identify descriptive information (stutter peaks, null alleles, large allele dropout), fragment loci were run through Micro-checker Version 2.2.3 (van Oosterhout et al., 2003).

Statistical Analysis

Fragment survival and TLE data were analyzed using R statistical software (R Core Team, 2017). Various survival and growth plots were created through the package 'ggplot2' to examine raw data (Wickham, 2016). A Survival Analysis (Cox model) was run to test if the independent variables of taxa, genotype, fragment section, and nursery site affected total colony mortality in the nursery (Therneau and Grambsch, 2000; Therneau, 2015; Kassambara and Kosinski, 2018). In addition to a survival analysis including all months, a survival analysis was run without the first month to test if there were differences in the factors affecting mortality due to collection, transport, and acclimation to the nursery.

For all growth analyses, data was analyzed up to 12 months, due to the loss of most fragments at site N1 at 13 months. For all fragments, linear extension (mm) was calculated as the total length measurement along the main axis plus the length of all branches >10 mm. This was then multiplied by partial survivorship (%) estimates to get Total Linear Extension (TLE) (mm) of live coral tissue. A Kendall's tau correlation was done to examine if the number of branches correlated to an increase in TLE. To test differences between changes in linear growth for surviving fragments (Growth = final-initial TLE) at 12 months, a Kruskal Wallis rank sum test was conducted, followed by a *post hoc* pairwise comparison using the Wilcoxon Rank sum test. An ANOVA was also conducted followed by a Tukey's HSD *post hoc* test to confirm factor differences.

To model the response of growth over time as a function of the independent categorical variables of taxa, fragment section, and nursery site, a Generalized Additive Mixed Model (GAMM) was run on the TLE data (Wood, 2011). Genotype, temperature, and light intensity were excluded from the models due to low sample size or the addition of a confounding factor to the model. Once fragments had died, they were excluded from dataset at the time in which they had died. Important terms were identified by backward selection (i.e., each term was sequentially dropped from the full model in turn) using Akaike's Information Criteria (AIC) scores. The final GAMM was then termed the Minimally Adequate Model (MAM) and was used for resulting analysis and *post hoc* tests. The MAM was validated by visual examination of the model residuals versus fitted values using plot(gam model) and gam.check(gam model) functions. Model validation did not indicate any problems based on residual plots. Pairwise comparisons on factor levels were then conducted using the "emmeans" package (Lenth, 2019).

As *A. palmata* has a more planar structure compared to *A. cervicornis* and *A. prolifera,* an analysis was conducted to compare average growth rate per month. For each surviving *A. palmata* fragment at 12 months, the total length*width

measurements were multiplied by two and then by partial survivorship (%) estimates to determine live fragment tissue sizes used in the growth rate equation. Width measurements were taken at the widest central point of the fragment, not including branch extensions. Photographic analysis of initial versus final width was completed for a subset of surviving *A. palmata* fragments (n = 12, minimum of 3 fragments from each site) to determine if width changed significantly during the full 13-month experimental period. Further description of the growth rate analysis and width comparison can be found in the **Supplementary Materials**.

When comparing values of new linear growth (mm/12 mo) for each fragment between genotypes, a Kruskal Wallis chi-squared test was used. To test differences between genotypes within a taxon, a One-Way ANOVA (parametric) or Kruskal Wallis chi-squared test (non-parametric) was used. If data met parametric assumptions, a Tukey's test (Tukey HSD) was used in *post hoc* analysis and visualized using the "multcompView" package (Graves et al., 2015).

To test differences in prevalence of conditions, a Kruskal-Wallis chi-squared test and Pearson's chi-squared test were used and data was visualized using the "pgirmess" (Giraudoux, 2018) and "vcd" (Zeileis et al., 2007) packages in R Studio. Conditions examined were bleaching (Blch), paling (Pale), and algal overgrowth interactions (OGA). No other conditions (disease/predation) were reported with enough replicates to be used in analysis.

Average daily temperature and average daily light intensity was calculated in Excel using the HOBO® temperature logger data. Loggers were deployed in March 2018, November 2018, and March 2019. While the study period did not include March 2018, this data was included in the light intensity analysis to increase the sample size and document site variability. Daily temperature data was calculated across the whole study period (June 2018–July 2019). Light intensity was measured in lux (lumen/ft^2). Photosynthetically active radiation (PAR) is typically used for light measurements, but to convert from lux to PAR a calibration curve and equation must be generated for each individual logger using a PAR meter (Long et al., 2012), which was not available for comparison. Light intensity was recorded throughout the study period, but due to biofouling of the sensor only the first week of light data after logger deployment was used in statistical analysis. Data was organized in Excel, imported into R, and analyzed with a Kruskal Wallis *t*-test and resulting *post hoc* analyses to determine whether the nursery sites had significantly different temperatures over the study period.

RESULTS

Survival and Mortality

Of the initial 157 fragments, 66 (42%) survived to the end of the study period (13 months). Of those fragments surviving at 13 months, 28.8% were *A. cervicornis* fragments, 31.5% were *A. palmata* fragments, and 39.7% were *A. prolifera* fragments. During the first month in the nursery (June 2018), overall total mortality was 29.3% (mortality being defined by fragments with no living tissue, not partial tissue loss). After the first month, monthly mortality ranged from 0 to 5% to July 2019. In July 2019, 32 fragments died, equating to 32.7% of the overall remaining fragments. Mortality was greatest at site N1, where only one fragment (*A. palmata*, basal fragment) was alive by the end of the study period. Site significantly affected coral fragment survival (Survival Analysis Cox model, $z = -5.47$, p = 4.5e–08), with site N3 fragments showing the greatest survival throughout the study period (**Figure 4**). No other factors had a significant effect on survival. Site was also the only significant factor in the survival analysis when the first month was excluded (to account for any mortality due to transport stress) (Survival Analysis Cox model, $z = -5.161$, p = 2.46e–07).

Growth

Descriptive Statistics and Linear Growth Analysis

Over 12 months, total TLE (mm) increased by 15.8%. There were no significant differences in the sizes of the ∼50 mm sections at initial nursery setup (Kruskal-Wallis test, $p > 0.05$). For surviving corals, linear growth (final – initial fragment TLE) and the final number of branches were significantly positively correlated [Kendall's tau correlation, $z(97) = 7.4452$, $p = 9.678e^{-14}$]. The equation that best describes the relationship is: Growth (TLE) = 29.83 + (13.16*number of branches), where the number of branches changes depending on the individual fragment.

Factors included in the growth analysis based on TLE were taxa, site, and fragment section. When comparing growth (final - initial TLE) for surviving fragments, mean linear growth values did not significantly differ among factor groups (Kruskal Wallis rank sum test, $p > 0.05$). However, a *post hoc* comparison indicated *A. prolifera* had significantly greater average growth (102.5 mm/12 mo ± 14.4 SE), than *A. cervicornis* (35.6 mm/12 mo ± 7.9 SE) and *A. palmata* (47.4 mm/12 mo ± 7.2 SE) (Paired Samples Wilcoxon rank sum test, $p < 0.05$) (**Figure 5**). Apical fragments had the greatest average growth (78 mm/12 mo ± 4.2 SE), although not significantly different (Paired Samples Wilcoxon rank sum test, $p > 0.05$) compared to middle fragments (53.3 mm/12 mo ± 4.0 SE) and basal fragments (57.6 mm/12 mo ± 3.6 SE). Site N3 showed greater average growth of fragments (74.1 mm/12 mo ± 4.2 SE) compared to N1 (60.9 mm/12 mo ± 3.0 SE) and N2 (55.2 mm/12 mo ± 4.8 SE), although sites were not significantly different from each other (Paired Samples Wilcoxon rank sum test $p < 0.05$) (**Figure 5**). An ANOVA between factor groups suggested a significant effect of taxa on linear growth (Analysis of Variance: Taxa – df = 2, $F = 10.344$, $p = 0.000114$). Apical *A. prolifera* fragments at site N3 had the greatest mean growth based on TLE after 12 months (**Figure 5**).

For the growth rate analysis, apical *A. prolifera* fragments at site N3 had the greatest average growth rate per month (10.47%) compared to all other factor combinations, and *A. prolifera* average growth rate per month was significantly greater than the parental species (Paired Samples Wilcoxon rank sum test, $p < 0.05$). *Acropora palmata* width measurements found no significant difference between initial and final mean widths (Parametric two-sample *t*-test, $t = 0.154$, $df = 21.9$,

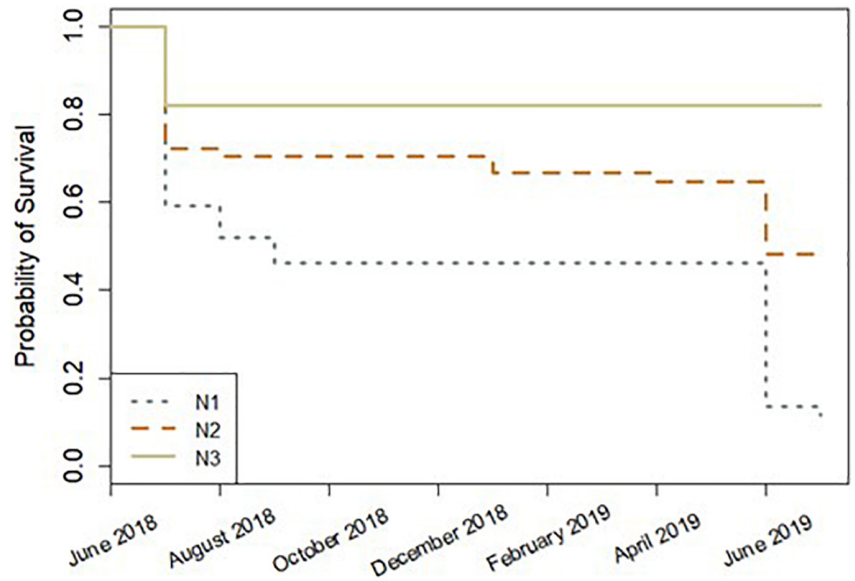

FIGURE 4 | Survival analysis plot by site for 13 months (June 2018–July 2019). Lower survival probability values indicate lower survival, and higher survival probability values indicate greater survival.

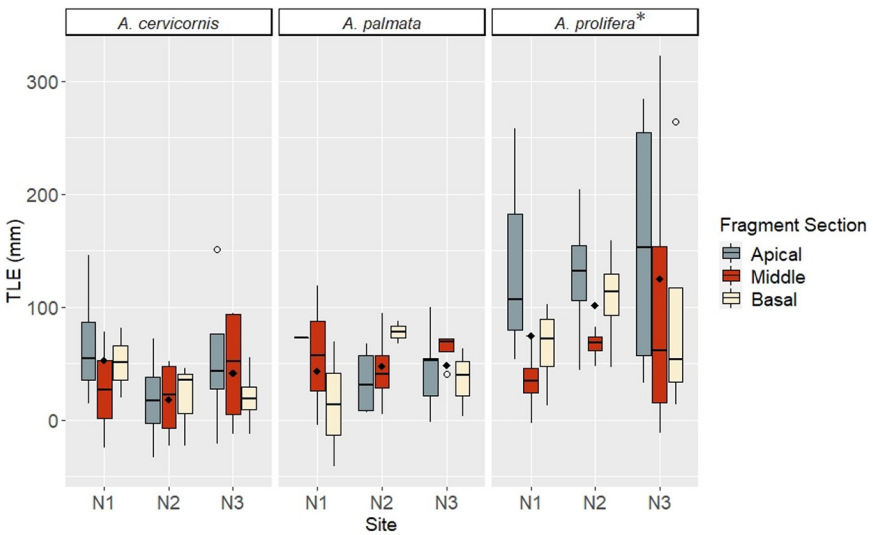

FIGURE 5 | Growth (TLE) by taxa, fragment section, and site at 12 months. Taxa are listed along the top bar of each plot. Site is listed along the x-axis within each taxa group. Fragment sections are differentiated by color in the legend on the right. Open circles indicate outliers; filled diamonds indicate mean values for the site. A * indicates significance.

p = 0.879). Mean change in width was 3.02 mm and ranged from 0.4 to 9.17 mm, with 90% between 0.4 and 4.58 mm. The **Supplementary Materials** contain further results of the growth rate and width analyses.

GAMM Analysis

Taxa, site, and fragment section were identified as important factors influencing growth over time (change in TLE, or mm/mo^{-1}) in the MAM (GAMM ANOVA, $p < 0.05$). The MAM included potential additive and interactive effects of factors that other statistical tests may not account for. To allow for dependency between individual fragments over time, initial size of each fragment was included in the TLE values across all time points.

Acropora prolifera fragments had significantly greater mean TLE values at 12 months compared to *A. cervicornis* and *A. palmata* fragments based on the MAM *post hoc* pairwise comparison ($p < 0.05$). *Acropora prolifera* fragments also had the greatest average monthly linear growth across all fragment sections and sites (**Figure 6A**). Based on the MAM, apical

and basal fragment TLE values at 12 months were significantly greater than middle fragments ($p < 0.05$) (**Figure 6B**). The MAM indicated apical *A. prolifera* fragments at site N1 had the greatest TLE (mm) at 12 months (June 2019), before most fragments at N1 died the next month (July 2019). However, 12-month TLE values between sites were not significantly different ($p > 0.05$) (**Figure 6C**).

Genotype

Genetic analysis confirmed there were five unique *A. cervicornis* and *A. palmata* genotypes, and 4 unique *A. prolifera* genotypes. Micro-checker found no evidence of scoring errors due to stutter peaks, large allele dropout, or null alleles across the five loci for each of the three taxa. Genotype did not have a significant effect on survival (Survival Analysis Cox model, $p > 0.05$). When analyzing the different genotypes of each taxon individually, genotype had a significant effect on linear growth values between taxa (Kruskal Wallis rank sum test, chi-sq = 33.3, $df = 11$, $p = 0.00048$). Genotypes within *A. cervicornis* were not significantly different from each other (Tukey HSD, $p > 0.05$). Within *A. palmata*, the mean linear growth after 12 months in genotype P10 was significantly greater than P1 and P11 ($p = 0.021$ and $p = 0.049$, respectively). All other genotypes within *A. palmata* were not significantly different from each other (Tukey HSD, $p > 0.05$). Genotype did not have a significant effect on linear growth values within *A. prolifera* genotypes (chi-sq = 0.72, $df = 2$, $p > 0.05$).

Conditions

Disease was not observed during the study period. Condition prevalence did not significantly differ over time (chi-sq = 3.1137, $df = 11$, $p > 0.05$). Condition type significantly affected prevalence (chi-sq = 22.28, $df = 2$, $p = 1.453e^{-5}$). Prevalence of bleaching was significantly lower than algal overgrowth interactions (OGA) and paling (Multiple comparison test, $p < 0.05$). There was a significant association between condition and taxa (chi-sq = 16.818, $df = 4$, $p = 0.002097$). Prevalence of OGA was less than expected in *A. palmata*, and prevalence of paling was more than expected in *A. palmata*; all other combinations of taxa and conditions occurred as expected. There was not a significant association between site and condition (chi-sq = 5.4002, $df = 4$, $p = 0.2486$), i.e., all combinations of condition and site occurred as expected.

Temperature and Light Results

During the study period, water temperatures ranged from 21.2°C to 33.5°C across all sites. Water temperatures at N1 ranged from 21.2°C to 33.5°C, at site N2 from 22.5°C to 32.3°C, and at N3 from 21.8°C to 32.9°C. Water temperature did not significantly differ between sites (Kruskal-Wallis test, $p > 0.05$), although site N1 had the greatest number of days (134) above a published bleaching threshold (29.8°C, Manzello et al., 2007) compared to sites N2 and N3 (123 and 126 days, respectively). Summer months (June–September 2018, June–July 2019) were compared specifically to determine if there were significant temperature differences in times of potentially greater heat stress. In the summer months, water temperatures ranged from 29.01°C to 31.72°C in 2018 and from 27.98°C to 33.45°C in 2019. When comparing just the summer months, there were no significant differences in mean temperatures between sites in 2018 and 2019 (One-way ANOVA/Kruskal-Wallis test, $p > 0.05$). There were six periods in the summer months where seven or more consecutive days were >29.8°C. Temperature stress based on Degree Heating Weeks (DHW) was calculated, which considers the number of days the sea surface temperature is ≥1°C above the approximate mean summer maximum temperature over a 12-week period (Wellington et al., 2001; Liu et al., 2006; Kayanne, 2017). For this study one DHW is equal to seven days ≥30.8°C. At all sites in July 2018 there were 8 to 9 consecutive days above this threshold, corresponding to 1 DHW. At sites N2 and N3 in June 2019, 7 consecutive days were above the threshold, also corresponding to 1 DHW. In July 2019, all but one coral fragment had died at site N1, likely attributed to bleaching.

Daily light intensity was variable between sites, although the variability was not consistent across logger deployments. At one week after initial logger deployment (March 2018), daily average light intensity ranged from 438.65 to 1434.47 lumen/ft^2 across all sites but was not significantly different between sites (Kruskal-Wallis, $p > 0.05$). In November 2018, daily average light intensity ranged from 145.83 to 459.75 lumen/ft^2 across all sites one week post-deployment and was significantly greater at site N2 than N1 and N3 (Kruskal-Wallis rank sum test, chi-sq = 9.719, $df = 2$, $p = 0.00775$; Paired Samples Wilcoxon rank sum test, $p < 0.05$). Sites N1 and N3 did not significantly differ from each other after the second logger deployment (Paired Samples Wilcoxon rank sum test, $p > 0.05$). At one week after the third logger deployment (March 2019), daily average light intensity ranged from 387.5 to 8987.47 lumen/ft^2 across all sites. Daily average light intensity one week after the third deployment was significantly different between sites (Kruskal-Wallis rank sum test, chi-sq = 12.445, $df = 2$, $p = 0.00198$), where light intensity was significantly greater at site N3 than sites N1 and N2, and light intensity at site N1 was significantly lower than N2 (Paired Samples Wilcoxon rank sum test, $p < 0.05$). Site N1 had lower light intensity than N2 and N3 in March 2018 and 2019 and had lower intensity than site N2 in November of 2018.

DISCUSSION

This study is the first to examine differences in survivorship and growth between the parental and hybrid taxa of Caribbean acroporid corals in a nursery setting, along with differences among fragment sections and nursery locations. While there are reservations about using hybrids in coral restoration due to genetic swamping concerns, the benefits of including fast-growing hybrid coral to quickly increase reef structure likely outweighs the potential long-term drawbacks. This study identified three main findings that will be beneficial to restoration management: (1) the hybrid taxon, *A. prolifera*, demonstrated greater growth in a shallow water nursery setting than the parental species, (2) using non-apical fragments did not compromise survival or growth, and (3) nursery site selection plays an important role in coral fragment survival.

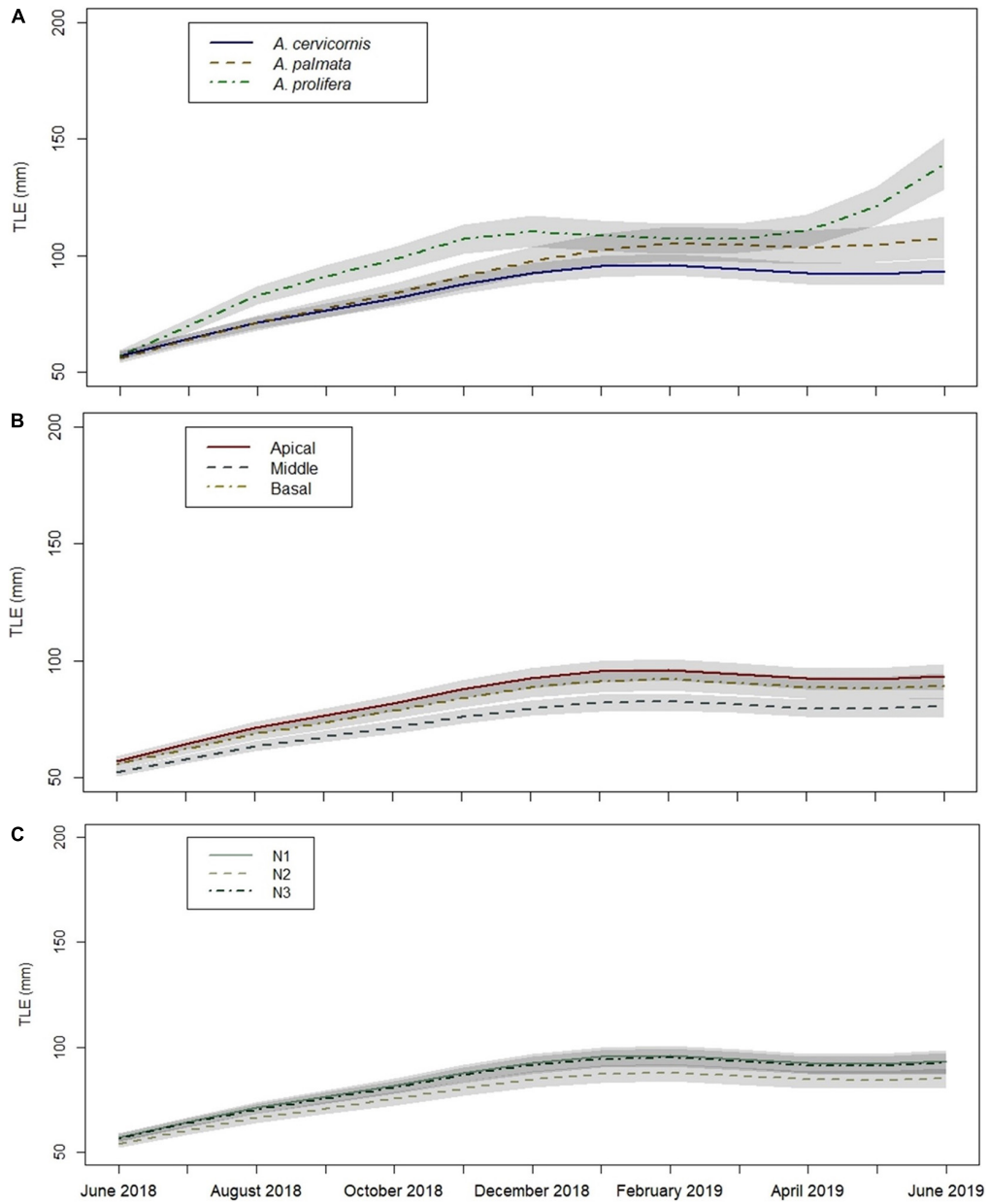

FIGURE 6 | Growth (TLE) over time based on Minimally Adequate Model (MAM) by: **(A)** taxa, **(B)** fragment section, and **(C)** site. Factor variables are given in each figure legend, differentiated by line type and color. Gray shaded areas denote standard error. Site N1 underlies N3 in Figure **(C)**.

There is growing evidence to suggest the *A. prolifera* hybrid has similar, if not higher, fitness than the parental species and may be a faster growing taxon overall (Gladfelter et al., 1978; Fogarty, 2012; Howe, 2018; Weil et al., 2020; Nylander-Asplin et al., 2021). This study provides further evidence that *A. prolifera* grows faster than the parental species. Our results found that the number of branches correlates with increased TLE over time, which is consistent with Lirman et al. (2014) and may explain why the prolifically branching hybrid had greater growth. As seen in other studies (Gladfelter et al., 1978; Crossland, 1981; Scheufen et al., 2017), growth of all taxa fluctuated seasonally and was greater in warmer months than in the cooler winter/spring months. Prior research has investigated the growth of wild acroporid coral colonies, where growth rates were higher in certain *A. prolifera* genotypes compared to *A. cervicornis* (Bowden-Kerby, 2008). In contrast, linear growth rates in *A. cervicornis* were higher than in *A. prolifera* in Puerto Rico (Weil et al., 2020), suggesting colony growth may be highly dependent on site location, environmental conditions, and genotype. With its rapid growth and prolific branching morphology, the hybrid is likely to reach outplanting goals by quickly increasing coral biomass and reef structure, albeit the

fused branches of the hybrid taxon may provide a different ecological service than the parental species. For example, the structure of *A. palmata* serves as a place for larger fish and invertebrates to live and hide. In contrast, the hybrid's fused branches are more compact, and may be more beneficial to the smaller fish and invertebrates.

Wild hybrid colonies have been found in greater abundance and in better health than their parental counterparts at some Caribbean sites (Fogarty, 2012; Hernández-Fernández et al., 2019; Nylander-Asplin et al., 2021). In surveys across the Caribbean, hybrid disease prevalence was equivalent to *A. palmata* and less than *A. cervicornis* (Fogarty, 2012). Despite hybrids often inhabiting shallower habitats (∼1 m) than the parental species, hybrids had comparable prevalence of paling or bleaching, which was low overall at surveyed sites (Fogarty, 2012). These characteristics, combined with the equal survivorship and rapid growth seen in our study, make the hybrid an ideal candidate to scale-up restoration. Addition of the hybrid could increase reef structure while also increasing the probability of genetic diversity within taxa (Willis et al., 2006; Richards and Hobbs, 2015; Nylander-Asplin et al., 2021). Hybrids could provide shallow water habitat with limited bleaching and paling, fast growth, and potentially less susceptibility to disease compared to one or both parental species, as our work and previous research indicates.

There is concern that the hybrid may outcompete the parental species or reduce genetic diversity if included in restoration practices, as seen in other research in forestry practices (Merkle et al., 2006; Richards and Hobbs, 2015; Kovach et al., 2016). However, concerns about genetic swamping of the parental species on evolutionary scales must not outweigh the immediate ecological need for shallow coral reefs, particularly when the state of coral reefs is dire. Genetics also play an important role in a coral's resistance to climate change and disease (Baums, 2008; Vollmer and Kline, 2008; Drury et al., 2016, 2017; O'Donnell et al., 2017; Baums et al., 2019; Chan et al., 2018, 2019a,b). With the inclusion of the hybrid, there is potential for greater sharing of genetic material across the three acroporid taxa via backcrossing, which may improve the adaptive potential of coral populations (Baums et al., 2019). Chan et al. (2019a) details a decision tree to address if/when a hybrid should be used in conservation efforts overall. To specifically address *A. prolifera* concerns, pilot studies could investigate differences in growth and survival of nursery grown coral by outplanting fragments in the same area in separate clusters, with enough separation between colonies to reduce competition between coral taxa. While there is overlap in habitat range among Caribbean acroporid taxa (Fogarty, 2012), further separation by habitat type/depth could help address competition concerns. This could include outplanting *A. prolifera* to shallow back reef areas, *A. palmata* along reef crests, and *A. cervicornis* to deeper reef slopes.

Apical fragments displayed the greatest TLE increase compared to middle and basal fragments, with the apical hybrid fragments having the greatest growth overall. Because these fragments were at the tips of the donor colony and contained the apical polyp, they were the primary location of growth on the original colony (Gladfelter et al., 1989; Rinkevich, 2000; Bowden-Kerby, 2001). This supports the idea that collecting from the tips of donor colonies may lead to a faster rate of growth, while also reducing impact to the donor colonies (Rinkevich, 2000; Bowden-Kerby, 2001; Herlan and Lirman, 2008). Previous studies have demonstrated gradients along *A. cervicornis* branches, where carbon compound transport was allocated toward the tips of colonies (Taylor, 1977) and respiration was higher in the terminal tips of *A. palmata* colonies (Gladfelter et al., 1989). The results of these studies indicate that the tips of acroporid colonies are areas of increased growth, where metabolic rates may be greater compared to the rest of the colony (Taylor, 1977; Gladfelter et al., 1989). In this study, there were no significant differences in linear growth by fragment section, which could be accounted for by branching on both cut margins of the middle and basal sections. Some studies have found that pruning of larger colonies of branching corals in a nursery leads to increased productivity after one year (Lirman et al., 2014), and similar exposure in massive corals by microfragmentation has led to a greater increase in tissue compared to singular colony (Page et al., 2018). In contrast, excessive pruning may lead to an increased risk of disease/overgrowth or reduce reproductive capability in the long-term (Epstein et al., 2001; Muller and van Woesik, 2012). Here, middle and basal fragments had two areas of recent exposed tissue from initial fragmentation, which can lead to increased risk of disease and other deleterious stress responses (Muller and van Woesik, 2012). Lesion colonization by opportunistic settlers, like algae, may affect the long-term growth of nursery fragments. In this study, initial algal settlement on the exposed coral skeleton was observed across all sites in the first month of nursery placement before the coral had an opportunity to heal. While no disease was observed on nursery fragments in this study, open or overgrown lesions may have contributed to partial mortality, leading to differences in growth between fragment sections. Further investigation of metabolic and chemical differences within a colony are needed to understand the role fragment section may play in nursery expansion and outplanting.

Site selection has proven to be an important factor in the success of coral nurseries, with temperature, water quality, and depth affecting survival (Shafir et al., 2006; Johnson et al., 2011; Young et al., 2012). The high mortality observed during the first month was likely due to transportation and acclimation stress. Transportation stress is difficult to avoid but can be reduced by multiple water changes and temperature control, if available. Storm and severe weather conditions may increase the risk of impact on nursery sites (Bowden-Kerby, 2001; Young et al., 2012), though previous research indicates that establishing nursery sites in areas with increased water flow may allow for higher survival (Edwards, 2010). Here, the site with the greatest survival and average growth, N3, was located on the unprotected northern side of the island. While site N1 fragment growth did not significantly differ from the other sites, it did have the lowest overall survival. Although we were unable to measure hydrodynamics at the study sites, we did observe stagnant conditions at site N1, likely contributing to the bleaching and subsequent mortality at this site in July 2019. Survival at site N2 was lower than N3 but

greater than N1, possibly due to increased water flow from the channel near this nursery location. Temperature and depth were consistent across all sites; therefore, it is likely that other environmental conditions, while not directly measured in this study, influenced survival and growth. Research by Nakamura and van Woesik (2001) demonstrated that branches in *Acropora digitifera* survived better in increased water flow conditions, even when exposed to higher water temperatures. Similarly, natural colonies and outplants of *A. cervicornis* had greater survival and abundance in areas with moderate to high water flow (D'Antonio et al., 2016; van Woesik et al., 2020). In this study, the negative impacts of stagnant water and increased water temperatures at all sites during the summer months likely outweighed the benefits of a more protected location. As suggested by Schopmeyer et al. (2017), survivorship < 80% over 12 months after collection may be due to poor nursery locations or genotypic differences. While Schopmeyer et al. (2017) compared *A. cervicornis* fragments, the same benchmarks could be applied for all taxa used in this study, and as such it is likely that site differences contributed to the less-than-ideal survival.

The light intensity data recorded after the third deployment (March 2019) shows differences between sites, with site N1 having the lowest intensity. Site N1 was located nearest to seagrass beds but in fine sediment compared to the other two nursery locations. It was the site that observationally had the greatest turbidity across the study period, which aligns with the low lux (lumens/ft^2) values from the light logger data. However, it is difficult to extrapolate this information further into the summer months. PAR is a useful metric to determine the ideal light intensity for photosynthesizing organisms, such as coral symbionts. Unfortunately, the lux data collected in this study requires direct calibration with a PAR meter, which was not available for comparison. As such, the light intensity data was used only as a secondary indicator of environmental conditions after temperature in this study. Turbid conditions may reduce the impact of irradiance on coral health (Wagner et al., 2010; van Woesik et al., 2012; Morgan et al., 2017; Sully and van Woesik, 2020; van Woesik et al., 2020). Other research has shown connectivity between adjacent seagrass beds and coral reefs via fish species and particulate matter (Dorenbosch et al., 2005; Heck et al., 2008), which could lead to increased food/nutrient supply (and therefore growth) for nursery fragments. However, high sediment input and long-term turbidity can increase prevalence of disease and other stressors to corals, likely impacting long-term growth and survival (Pollock et al., 2014; Ng et al., 2016).

Overall, growth is only a secondary measure of success if nursery fragments do not survive. As such, site selection based on survival alone should be a priority before considering growth. Site selection criteria should consider depth, water temperature, site accessibility, hydrodynamics, and nutrient flux in the area, which could be evaluated using smaller pilot studies. Locations with optimal depth, moderate water flow, adequate light attenuation, and a limited range of temperatures will likely lead to the most successful coral fragment survival and growth (Edwards, 2010; Johnson et al., 2011).

CONCLUSION

The hybrid coral utilized in this study showed greater fitness than the parental species. Coral restoration managers should consider the fast-growing hybrid *A. prolifera* as an option for restoration. The hybrid survives as well as and grows faster than the parental species, and as such is a potential option to increase shallow reef infrastructure through restoration. Including the hybrid taxa and increasing the number of unique parental genotypes in a nursery will increase genetic diversity among all three taxa in future restoration activities. As shown in this study, evaluating appropriate nursery sites before setup is crucial to project success. Although apical tips of colonies prove to be a source for fast growing tissue, further research is needed to confirm there are no tradeoffs between growth and survival. Finally, our study took place over the course of one year at a remote island in the Bahamas. Incorporating the hybrid in different aspects of active restoration at sites throughout the Caribbean or at larger scales would help determine how this taxon fits into the larger picture of coral restoration.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to authorization required by the program sponsor. Upon approval, data will be provided by the corresponding author. Requests to access the datasets should be directed to CV, cassie.vanwynen@gmail.com.

AUTHOR CONTRIBUTIONS

CV, MH, and NF designed the study. CD obtained permitting for the project. CV, MH, NF, and CD scouted and set up nursery sites. CV, MH, and CD collected corals for the nurseries. MH and CV were responsible for project logistics, data collection, and nursery maintenance, with assistance from NF and DG, and conducted genetic processing and analysis. NF and DG were responsible for project budgeting. CV organized and analyzed the data and wrote the manuscript drafts. CV, MH, NF, and DG contributed to interpretation of results. All authors contributed to final draft edits before submission.

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participate in the data analysis, interpretation, or in the writing of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.669966/full#supplementary-material

Supplementary Figure 1 | Coral collection locations around New Providence, shown by the yellow points. *Inset*: New Providence (in box) in relation to Florida and the Berry Islands.

Supplementary Figure 2 | Nursery site experimental setup. Coral fragments were placed on the middle three tree branches shown here with a letter and number value. Fragment sections were individually tagged. C, P, and H denote taxa (*A. cervicornis, A. palmata,* and *A. prolifera* (hybrid), respectively), and numbers denote genotype. Not all putative genotypes were unique, and so some genotypes had a higher number of fragments after analysis. The fragment section changed branches between trees at each site. Dashes denote no fragment attached.

Supplementary Figure 3 | Growth (TLE) over time by genotype. TLE (mm) is along the y-axis, and time is along the x-axis. C, P, and H denote *A. cervicornis, A. palmata,* and *A. prolifera* (hybrid), respectively.

Supplementary Figure 4 | Frequency analysis of conditions present across the study period by taxa. Conditions are listed along the top x-axis, with taxon listed along the y-axis. Algal overgrowth, bleaching, and paling are denoted by OGA, Blch, and Pale, respectively. Blue boxes indicate that the condition occurred more than expected for a specific taxon, while red indicates the condition occurred less than expected. Gray boxes indicate that a condition occurred as expected for that taxon.

Supplementary Figure 5 | Mean daily temperature by site from June 2018 to July 2019. Purple line denotes published approximate bleaching threshold at 29.8°C (Manzello et al., 2007). Sites are differentiated by color, shown in the legend.

Supplementary Table 1 | Linear growth (TLE) at 12 months by genotype. C, P, and H denote *A. cervicornis, A. palmata,* and *A. prolifera* (hybrid), respectively. Genotype with * indicates only 3 fragments left at 12 months.

Supplementary Table 2 | Monthly temperature ranges (°C) from June 2018 to July 2019.

Supplementary Table 3 | GPS coordinates for nursery locations.

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BRIEF RESEARCH REPORT
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Capture, Culture and Release of Postlarvae Fishes: Proof-of-Concept as a Tool Approach to Support Reef Management

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The changing world presents negative impacts on marine ecosystems and has led to the development of diversified tools to support reef restoration. Harnessing restoration to achieve success needs innovative techniques that also address the restoration of reef fish assemblages, contributing to the conservation of biodiversity and ecosystem functions and also tackle the cost-effectiveness through impact-driven solutions. Here, we propose a proof-of-concept for enhancing fish populations on reefs using: (1) postlarvae capture, (2) aquarium culture, and (3) release to reef sites. We conducted field studies in the Mexican Caribbean to analyze for the first time, the possibility of using the capture and aquarium culture of postlarvae fish species and release of juveniles as a tool for the potential recovery of reef biodiversity resilience. We tested the potential of postlarvae capture using two distinct night light traps (BOX and collect by artificial reef ecofriendly traps, C.A.R.E.) in three sampling sites with different distances from shoreline and depth. We collected 748 postlarvae reef fishes from eight orders, 20 families, and 40 species. *Acanthuridae*, *Pomacentridae*, *Monacanthidae*, and *Tetraodontidae* comprised the highest species number of postlarvae families. We also set up a pilot release experiment with *Stegastes partitus* using two trials (32 and 1 day after capture) and propose analysis to determine appropriate reef sites to release the cultured juveniles and to aid ecological planning. We present the results of the pilot release experiment with *S. partitus*, showing that there is a positive effect in survivorship during the capture (80%) and release (76–100%) procedures into suitable habitat and good chance that more studies will bring novelty to the field. Although trials carried out with more species relevant to restoration will be needed. The use of these techniques can be a great opportunity to improve the research of restoration efforts in the Caribbean region with fish-depleted coral reefs with vulnerable food webs, especially at local scales and supporting other management strategies.

Keywords: active restoration, biodiversity, Caribbean, coral reef, fish restocking, recovery

INTRODUCTION

Active strategies approach has been proposed (Schmidt-Roach et al., 2020) to promote reef restoration in areas with severely decreased reef fish communities (Abelson et al., 2016). In the Caribbean, restoration efforts have been focused on the recovery of corals (Bayraktarov et al., 2020; Calle-Triviño et al., 2020). Although social and ecological outcomes target various benefits (Calle-Triviño et al., 2018), current challenges of these efforts include long-term implementation, identifying the feasibility of this implementation, and ecological processes restoration (Ladd et al., 2018; Duarte et al., 2020).

Efforts for broadening the reef restoration from coral species to fishes remain limited and have been rarely used as a method (Obolski et al., 2016). Nevertheless, reef fishes have a pelagic larval phase during their lifecycle that allows them to disperse spatially and a demersal juvenile phase that leads to the colonization of these species on a reef (Sale, 2015). The former is one of the most critical stages in their cycle, which determines the characteristics of the populations such as distribution, abundance, and population dynamics (Victor and Wellington, 2000; Simpson et al., 2013). It is a phase where they experience high-mortality rates of ∼60% (Doherty et al., 2004; Almany and Webster, 2006), and survival is often related to early life-history traits (Sponaugle et al., 2011). This postlarval phase continues to be studied to understand the settlement processes (Dufour, 1994; Hendriks et al., 2001; McCormick et al., 2002; Lecaillon, 2017). Several studies have used settlement stage reef fishes to realize small-scale fisheries based on postlarval capture and culture (Bell et al., 2009). This method has also been integrated into experimental protocols as a potential tool for restoration of fish assemblages as a proof-of-concept (Heenan et al., 2009; Abelson et al., 2016). The aforementioned is based on the concept of removing extremely high-mortality rates that occur during settlement, and post-settlement of the first few weeks, and take advantage of this process to significantly increase survivorship (Vallès et al., 2008).

Several techniques have been developed to capture coral reef fish in the early life stages (Choat et al., 1983), including light traps (Lecaillon, 2004; Moana Initiative, 2007), "hoa" nets that are used in shallow passes (termed hoa in French Polynesia) that allow water to enter "closed" and "semiclosed" atoll lagoons (Lecaillon and Lourié, 2007) and crest nets in French Polynesia (Dufour and Galzin, 1993), Australia (Doherty and McIlwain, 1996), Solomon Islands (Hair et al., 2002), and La Reunion (Durville et al., 2003). In addition, extensive light trap work has been carried out in the Florida Keys, focused on examining and measuring the processes of larval fish supply (Sponaugle et al., 2006; D'Alessandro et al., 2007) and capture during the settlement stage of *Stegastes partitus* larvae (Rankin, 2010). Remarkably, there have been very few studies on the postlarvae and early stages of ichthyofauna within the Caribbean coral reefs. Vásquez-Yeomans et al. (1998, 2003, 2011) and Álvarez-Cadena et al. (2007) carried out studies on ichthyoplankton obtained by surface trawling, and channel nets. Recently, Ayala-Campos (2014) used a light trap technique for sampling.

Despite these research efforts, little data have been collected on postlarvae of the fish in the Caribbean region, some of which have not been published. In addition, implementing these low-environmental impact techniques as an effective tool for biodiversity monitoring and conservation activities are absent. Given this situation, we performed a proof-of-concept to enhance fish populations on reefs by using techniques for postlarvae capture, aquarium culture, and release. In addition, the goal of this study was to supply primary information to address the current interest in coral reef restoration. To do so, we described temporal variations in postlarvae abundance of reef fishes during the high-settlement season by testing two different kinds of night light traps. We addressed the aquarium-culture factors and release of cultured juveniles, using a pilot release experiment with *S. partitus* and a landscape analysis. We use these results and analysis to test feasibility and comment on the potential ecological application of this method into Caribbean restoration efforts.

MATERIALS AND METHODS

Study Approach and Location

A primary criterion for applying our approach and address the study was to consider a multidisciplinary engage approach. It was necessary to establish a strong partnership among the science, public (marine-protected area; MPA), and private sectors (Xcaret and Wave of Change) (Abelson et al., 2016; Doropoulos et al., 2019). In this sense, operationalizing the study, the choice of study site, technical process setup, and field collection were conducted based on the requirements for feasible applications in the Caribbean region (**Figure 1**).

The study was performed within an MPA in the northern part of the State of Quintana "Reserva de la Biosfera del Caribe Mexicano." This location was chosen because: (1) the area is managed by the authorities (Comisión Nacional de Áreas Naturales Protegidas; CONANP); (2) the staff of Xcaret control the recreational activities including protection and fishing regulations; and (3) it has proximity to logistic facilities to support the study in all the stages. Sampling was conducted in the three sites: Site 1 (Punta INAH reef) is closer to the shore (100 m) with a depth of 10 m. This area is characterized by more complex coral reef structures in comparison with the other sites, the reef crest is conspicuous, and the area is covered with larger seagrasses. At Site 2 (Punta Venado reef), the distance to the coast is 200 m, with an average depth of 12 m, the bottom is mainly characterized by low-seagrass coverage and some isolated reef patches. At Site 3 (Calica reef), the distance to the shoreline is 300 m, with a depth of 15 m, dominated by a few isolated patches of seagrass and coral reef.

Device for Capturing Postlarvae and Methodology

Between July and October 2018, which are the months for the highest recruitment (Williams and Sale, 1981; Leis and McCormick, 2002; Watson et al., 2002; Ayala-Campos, 2014), monthly catches of postlarvae fish were conducted at all three sites as a part of the first stage method. These catches consisted

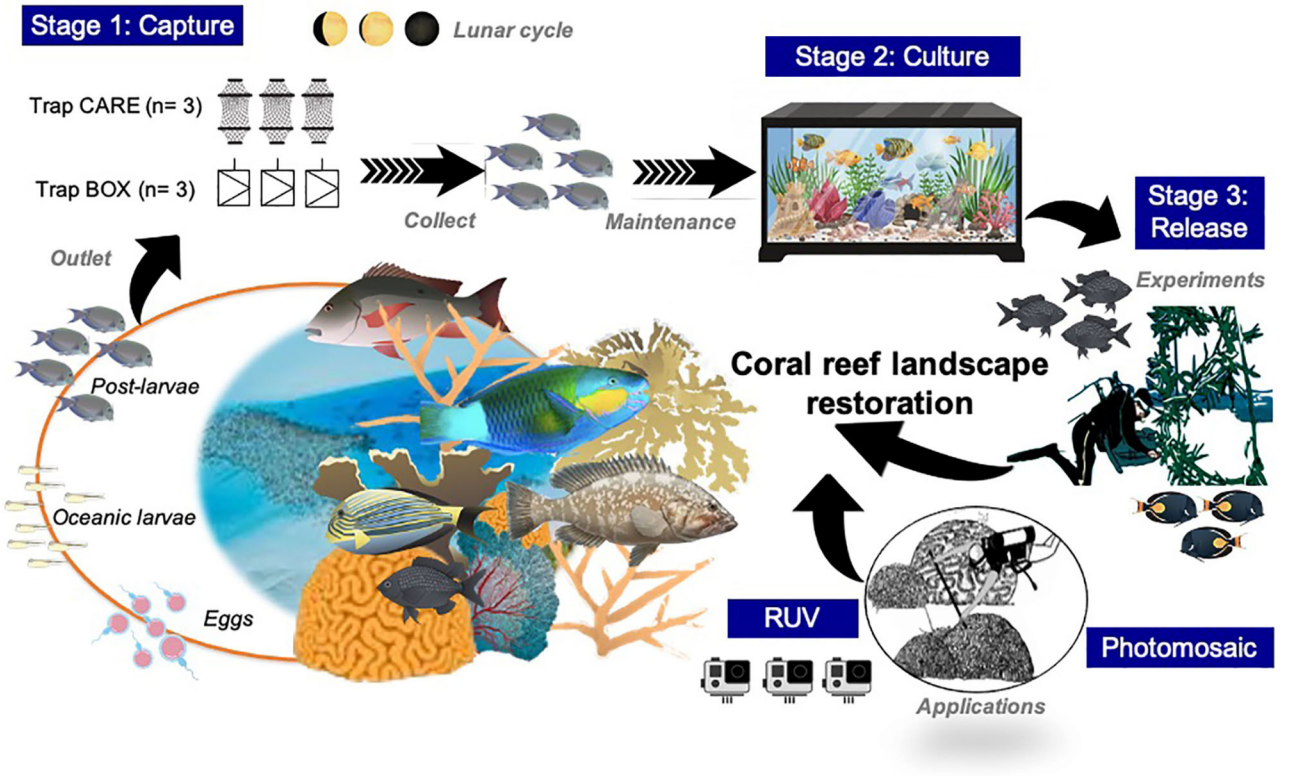

FIGURE 1 | General outline process for scaling up restoration of coral reef using oostlarval reef fishes: capture, culture, and release.

of the placement of six-night light fish traps in total ($n = 3$ replicates per treatment) at each site: three Australian design single chamber box (acrylic) traps on each side and three C.A.R.E design traps (Collect by Artificial Reef Ecofriendly patented by Ecocean) (Lecaillon, 2004) (**Supplementary Figure S1**). The traps were deployed during nights of minimal lunar illumination (new moon), set at sunset, and removed at sunrise (minimum of 10 h functioning) for 7 consecutive days.

Aquarium-Culture of Postlarval Fish

As a second stage, a total of 48 fish tanks (32 tanks × 10 L and 16 tanks × 20 L) were designed and built for the sorting, maintenance, and observation of captured organisms. The larger tanks were used to accommodate species of fish that were collected in larger numbers to prevent overstocking or hyperpredation in the tanks. On capture days, the fish were distributed in fish tanks according to the species or behavior (territorial, aggressive, and passive). The fish tanks were conditioned with shelters made of sheets of PVC and/or raffia fibers. The fish were fed twice a day (at 0,800 and 1,700 h) with nauplius, adult artemia (*Artemia* sp.), and commercially prepared food. The organisms observed were identified following the Cervigón et al. (1992), Humann and DeLoach (2002) identification guides and systematically sorted according to FishBase criteria (Froese and Pauly, 2019). All the collected postlarvae were monitored for 15 days post-capture (complete metamorphosis) to test for differences in survival (Victor, 1991).

Release of Cultured Juveniles Into Caribbean Reefs

To explore the role of releasing cultured juveniles as a feasible restoration tool, we considered site-specific survival, efficiency of target species behavior, and ecology of reef fishes. Given that habitat variation is a key modeling factor in the early-stage of reef fishes (Paddack and Sponaugle, 2008), we performed a pilot release study in a controlled matrix or artificial structure for the juveniles. As the first step of this stage, we selected individuals of bicolor damselfish (*Stegastes partitus*) for the experiment based on their prevalence, territorial behavior, and their fidelity to the habitat where they settle (Thiessen and Heath, 2006; Heenan et al., 2009). A total of 42 samples of *S. partitus* were collected and released using two trials to test the site survival of released fish: (1) postlarvae collected and cultured for a period of 32 days; and (2) juveniles collected and released 1 day after capture. Trials were equally split among 21 individuals, resulting in three replicates placed in six tanks (200 L) with seven individuals per tank, considering a 3 cm size for all the replicates. The tanks had artificial structures (matrix) made of cement, raffia, and ceramic tiles as refuges. Before the release of cultured juveniles, the structures were transported to the selected slopes (12 m depth).

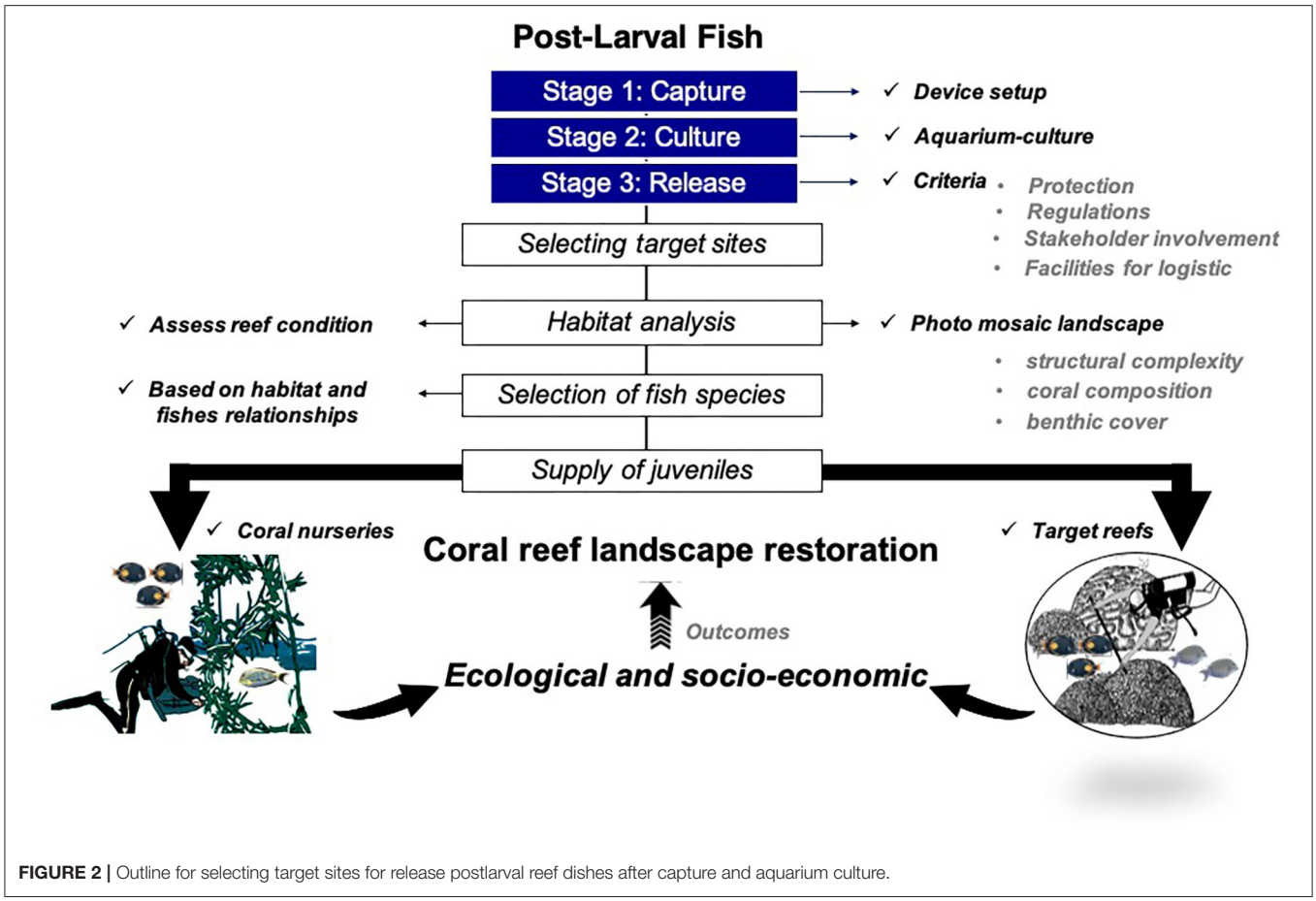

FIGURE 2 | Outline for selecting target sites for release postlarval reef dishes after capture and aquarium culture.

The artificial structures were haphazardly arranged on the slopes of reefs separated by 10 m between each structure to prevent migration between them. Release protocol and duration of visual counts were focused on to assess post-release survival. Based on the methodology used by Heenan et al. (2009), site survival of released fish was measured twice on days 1, 2, and 3 using visual census (at 0,800 and 1,700 h) and then once daily (0,800 h) for another 5 days, for a total of 8 days.

Selecting Target Sites for Release Criteria

Environmental requirements such as structural complexity, coral composition, fish community, and benthic reef cover need to be considered to enhance the release approach (**Figure 2**). We used the Agisoft Metashape Professional Edition software to process preliminary images that we obtained by taking pictures with two cameras (GoPro 8) for the building of the photomosaics in Cozumel (Francesita reef) and Riviera Maya (Manchoncitos reef). In addition, we carried out an assessment corresponding to fish communities already present.

Data Analysis

To recognize the temporal variations through species abundance, we evaluated species richness (number of species present in each sample) and Shannon-Weaver's diversity (H) (Jost, 2007). An analysis of the local contribution to beta diversity was used to determine the percentage of contribution in each of the sites and identifying the uniqueness based on existing diversity (Legendre and De Cáceres, 2013).

A Multivariate Permutational Analysis of Nested Variance (Nested PERMANOVA) was carried out to determine differences between capture months. The ANOVA one-way was used to determine differences between the catch per unit effort (CPUE) (individual/trap/day) of fish caught by the two different light night traps. A CAP was carried out to visualize the dispersion of the samples through a season with the same data matrix. A heat map (descriptive analysis) of the total abundances of postlarvae species captured by the months of study was made, with cluster analysis (similarity with Euclidean distances).

Differences in site survival of released fish among trials were compared with Kaplan-Meier's survival analysis, with CIs ($\alpha = 0.05$), followed by Log-Rank (Wilcoxon) pairwise comparisons. All the analyses were performed and plotted using the statistical program R version 3.3.1 (R Core Team, 2017).

RESULTS

A total of 748 postlarval reef fishes from eight orders, 20 families, and 40 species were identified (**Table 1**). The most abundant species were those belonging to the families *Acanthuridae*,

TABLE 1 | Taxonomic list of postlarval fish recorded in the northern Mexican Caribbean.

Order	Family	*Species*	Capture method
Anguilliformes	Congridae	*Ariosoma balearicum*	BOX
Beloniformes	Exocoetidae	*Cheilopogon melanurus*	BOX
	Hemiramphidae	*Hemiramphus brasiliensis*	BOX
Beryciformes	Holocentridae	*Sargocentron vexillarium*	BOX, C.A.R.E.
		Sargocentron coruscum	BOX
Lophiiformes	Antennariidae	*Antennarius striatus*	C.A.R.E.
Perciformes	Acanthuridae	*Acanthurus tractus*	BOX, C.A.R.E.
		Acanthurus coeruleus	C.A.R.E.
		Acanthurus chirurgus	BOX, C.A.R.E.
	Apogonidae	*Paroncheilus affinis*	BOX, C.A.R.E.
		Astrapogon puncticulatus	BOX, C.A.R.E.
		Phaeoptyx pigmentaria	BOX, C.A.R.E.
		Apogon maculatus	BOX, C.A.R.E.
		Apogon aurolineatus	C.A.R.E.
	Carangidae	*Caranx latus*	BOX
	Chaetodontidae	*Chaetodon ocellatus*	BOX
		Chaetodon capistratus	BOX, C.A.R.E.
	Gerreidae	*Eucinostomus melanopterus*	C.A.R.E.
	Kyphosidae	*Kyphosus sectatrix*	BOX
	Lutjanidae	*Lutjanus apodus*	BOX
		Lutjanus griseus	BOX, C.A.R.E.
		Lutjanus analis	BOX, C.A.R.E.
	Pomacanthidae	*Holacanthus ciliaris*	BOX, C.A.R.E.
		Pomacanthus arcuatus	BOX, C.A.R.E.
	Pomacentridae	*Microspathodon chrysurus*	BOX, C.A.R.E.
		Stegastes partitus	BOX, C.A.R.E.
		Stegastes adustus	BOX, C.A.R.E.
		Abudefduf saxatilis	BOX, C.A.R.E.
		Stegastes leucostictus	C.A.R.E.
		Chromis cyanea	BOX, C.A.R.E.
		Stegastes variabilis	BOX
	Sphyraenidae	*Sphyraena barracuda*	BOX, C.A.R.E.
Pleuronectiformes	Bothidae	*Bothus ocellatus*	C.A.R.E.
Scorpaeniformes	Scorpaenidae	*Scorpaena inermis*	BOX, C.A.R.E.
Tetraodontiformes	Monacanthidae	*Monacanthus tuckeri*	BOX, C.A.R.E.
		Cantherhines pullus	BOX, C.A.R.E.
		Monacanthus ciliatus	BOX, C.A.R.E.
	Tetraodontidae	*Canthigaster rostrata*	BOX, C.A.R.E.
		Sphoeroides spengleri	BOX

BOX, Australian design of a single chamber box and a lower collector and C.A.R.E, collect by artificial reef eco-friendly traps.

Pomacentridae, *Monacanthidae*, and *Tetraodontidae*. The most dominant species were the bicolor damselfish (*Stegastes partitus*) with 132 individuals, followed by the sharpnose-puffer fish of the family Tetraodontidae (*Canthigaster rostrate*) with 121 individuals. The highest abundances and CPUE occurred in October (267 individual and 10 individual/trap/day), followed by August (227 individual and 8.36 individual/trap/day). The highest richness (taxa) and diversity (H') was recorded in August and September, and the lowest was in July (**Supplementary Figure S2**).

Species composition by month showed significant differences in terms of species replacement component of beta diversity ($p < 0.001$) (**Supplementary Table S1**). Canonical Analysis of Principal Coordinates confirms differences among months (**Supplementary Figure S3**). July and October were the months with the highest variation of species and least similar in terms of beta diversity, while August and September were more similar (**Supplementary Figure S4**).

Of the total of 748 individuals, each of the trap types collected approximately 50% of the total fish present (367 individuals in the box design light traps and 381 individuals in the C.A.R.E light traps). The species with the most significant contribution were: *S. partitus* (Family: *Pomacentridae*), *Canthigaster rostrata* (Family: *Tetraodontidae*), *Monacanthus tuckeri*, and *Cantherhines pullus* (Family: *Monacanthidae*) with a contribution of more than 50% of the total species composition (**Figure 3**). Species composition between the different months showed the highest abundance and presence of *S. partitus* and *C. rostrata* in all the months. However, most of the less abundant species were replaced month by month, for example, of the 23 species recorded in August 9 species were replaced in October.

A total of 367 individuals of 34 species were identified from the box light traps. Several rare and unique species were recorded in the net catches in this trap. Of these species, six presented only one individual (*Lutjanus apodus, Kyphosus sectatrix, Sargocentron coruscum, Hemiramphus brasiliensis, Ariosoma balearicum*, and *Caranx latux*), and two presented more than one individual (*S. variabilis, Sphoeroides spengleri*, and *Cheilopogon melanurus*). The C.A.R.E. light traps captured a total of 381 individuals of 30 species. Five species were unique to this trap and were rare with at least one individual per species (*Eucinostomus melanopterus, Bothus ocellatus, Apogon aurolineatus*, and *Antennarius striatus*). The species *Acanthurus coeruleus,* with an abundance of seven individuals, was caught only by the C.A.R.E. trap. Despite the observed differences in species composition by trap type, the species replacement component of beta diversity was not significant (PERMANOVA, 999 permutations: F1, 53 = 1.23, $p = 0.45$). However, the one-way ANOVA for CPUE variation between the two types of traps showed a significant difference ($P = 0.000676$) (**Supplementary Table S2**). The C.A.R.E. type traps obtained not only higher CPUE of postlarvae type but also more considerable variation in their records (12.4 individual/trap/day ± 5.54 CI) when compared with the box design traps (4.3 individual/trap/day ± 1.26 CI).

Survivorship results based on aquarium culture were analyzed using an overall approach. Survivorship of total postlarvae captured during the aquarium-culture stage was 80% until 15 days of post-capture. Also, we evaluated the survivorship for release stage, after 8 days of the site-specific survival experiment, and 76% of juveniles released within 1 day of capture ($n = 16$)

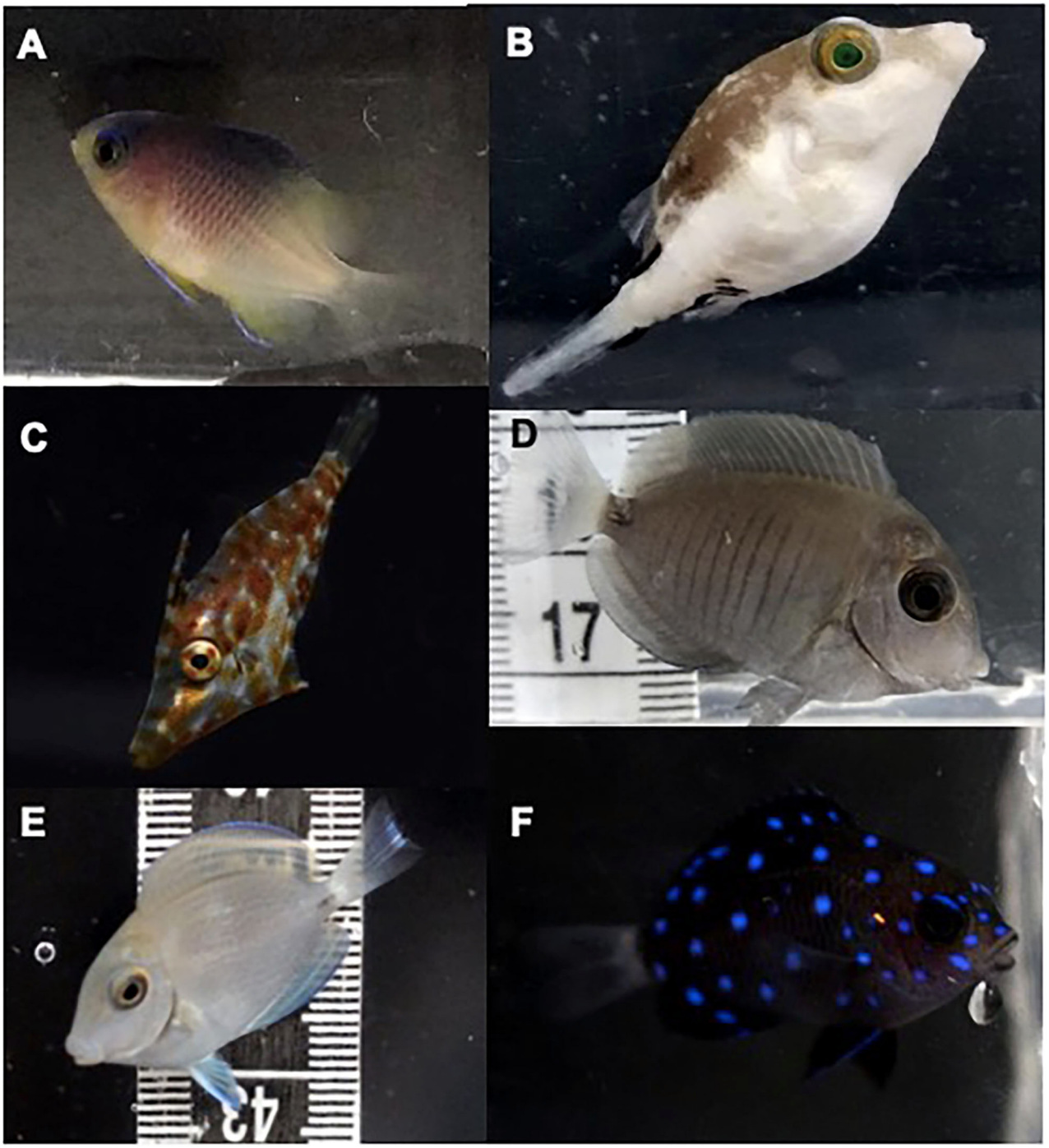

FIGURE 3 | Some of the most abundant species recorded in the area include the following: **(A)** *Stegastes partitus*; **(B)** *Canthigaster rostrata;* **(C)** *Monacanthus tuckeri*, **(D)** *Acanthurus chirurgus*, **(E)** *Acanthurus tractus*, and **(F)** *Mycrospathodon chrysurus*.

survived, while juveniles released after 32 days in culture survived 100% ($n = 21$). A significant difference in survival of juveniles released was evident based on results for the two trials ($P = 0.04$, $X2 = 4.2$).

Results based on the preliminary landscape analyses suggest different target sites for release criteria. For example, a seascape dominated by *Agaricia agaricites*, *A. tenuifolia*, *Porites porites*, and *P. astreoides* were present in Cozumel (Mexico) on a shallow reef (8–10 m). The reef-building corals (e.g., *Orbicella* complex and *Montastrea cavernosa*) were present in the Manchoncitos Reef (Riviera Maya) between 9 and 13 m depth range. The highest contributions of fish species in Francesita reef 27.6%

were covered by grunts, 11.8% by surgeonfishes, and 10.1% by parrotfishes, while in Manchoncitos reef were 43.6% were covered by grunts and 27.1% by parrotfishes. *Haemulon flavolineatum* and *H. aurolineatum* were the most species abundant in Francesita, while *Caranx ruber* and *H. aurolineatum* were in the Manchoncitos reef.

DISCUSSION

This study describes the method of capture using two kinds of night light traps, BOX (Doherty, 1987) and C.A.R.E (Ecocean, 2020). Even though this study is preliminary, results show some distribution patterns similar to fish postlarvae in a previous survey (Ayala-Campos, 2014). The species richness (40 spp.) is consistent with most of the representative families: *Acanthuridae*, *Pomacentridae*, *Monacanthidae*, and *Tetraodontidae*, in addition to the more abundant species *S. partitus* and *C. rostrata* (Álvarez-Cadena et al., 2007). Therefore, the species identified in this work constitute 40% of the taxonomic diversity for this region reported by Álvarez-Cadena et al. (2007). In previous studies, 118 species belonging to 53 families and 115 species belonging to 55 families were recorded, respectively (Vásquez-Yeomans et al., 2011). However, most of the research they conducted was not recorded as postlarvae.

The difference in species richness may be related to the method and effort of sampling (Cortés-Useche et al., 2018). In the previous studies conducted in the Mexican Atlantic, a greater number of sites and different sampling methods (i.e., trawls, dredge, and crest nets) were used (Del Moral-Flores et al., 2013). In addition, other studies have focused on the estimation of species richness in tropical communities through the use of various sampling gear and methods (Vásquez-Yeomans et al., 2011). Considering the biology of the species besides their positive phototactic response, capture with light traps has the advantage of efficiency in catching reef species which prefer sheltered locations (tigmotropism), while non-target pelagic fish just swim over the reef (Lecaillon and Lourié, 2007). However, in order to increase efforts in the context of reef restoration, the use of other types of traps such as hoa traps and crest nets may be considered.

The capture of postlarvae during the summer season observed here is consistent with the highest CPUE in French Caribbean islands (Lecaillon, 2017). Our study, focused on captures made, taking into account that most of the recruitment occurs during a relatively short period, during the summer months, and postlarvae have movement during dusk and at night which is more significant on nights with lower luminosity (Dufour and Galzin, 1993; Milicich and Doherty, 1994; Leis and McCormick, 2002; Watson et al., 2002). However, the differences observed between October and July could be due to peaks of temperature during the summer. Villegas-Sánchez et al. (2009) associate the differences in these peaks with variations in sea temperature.

The catches obtained by the two kinds of night light traps showed that the C.A.R.E. trap obtained higher abundances (CPUE), but a lower richness (four fewer species). The effectiveness of this method is because it uses an artificially lit space that takes advantage of the behavior of the recruits to catch them (Lecaillon and Lourié, 2007): (1) the attraction to light (phototropism); (2) the desire to come into contact with a solid object (tigmotropism); and (3) the need to take refuge from predators. These sensory elements are essential for fish postlarvae, which have very acute senses during recruitment (Sweatman, 1988; Kingsford et al., 2002; Lecaillon and Lourié, 2007). The differences observed in CPUE may be associated with the design of the two types of traps, the intensity and type of light (Vadziutsina and Riera, 2020). It is important to highlight the fact there is a risk of the occurrence of hyperpredation in and around the traps during the capture process. For example, in our study, we recorded the presence of predatory individuals of *S. barracuda*, and we recommend checking the traps continuously to avoid filling them with many individuals and predatory species. In addition, we recommend separating the captures early on in the boat by species and size, thus avoiding the loss of individuals and the loss of benefits of this method of local capture.

Despite the differences between the two types of traps, C.A.R.E and BOX traps can be considered for harvest in a complementary range of species, to catch a greater diversity and larger number of fish (Dufour et al., 1996). In this study, postlarvae collected (**Table 1**) through the C.A.R.E and BOX traps were effective in terms of both the diversity (40 species) and abundance (748 target postlarvae collected). These results illustrate that capture of postlarvae in the summer season in the context of feasibility is the most appropriate period for collection in the Caribbean region. Moreover, following sampling from three sites, the results of the abundance suggest that further upscaling of locations can provide a more significant number of captured organisms to improve culture and release stages.

In this study, the overall production of juveniles has been achieved successfully with almost 80% survival until 15 days post-capture. For other reef fishes, Durville et al. (2003) cited survival between 60 and 92%, and Moana Initiative (2007) reports survival of target fish grown out were 80% after reaching the juvenile size. Survival can be considered as a crucial criterion for evaluating the optimum-rearing condition. Evidence from experimental studies with reef fishes (Planes and Lecaillon, 2001; Steele and Forrester, 2002; Webster, 2002; Doherty et al., 2004) suggests an advantage in the culture of postlarvae in contrast to postlarvae that settled in the wild. These experience high mortality as a result of increased predation rates (Bailey and Houde, 1989; Doherty, 2002). This result is significant for future directions of scaling up production as it allows for feasibility in its implementation. Overall, technical knowledge of aquarium culture must be expanded, for example, tests are needed to compare survival rates post-capture of specific species and functional groups (i.e., based on trophic level).

To our knowledge, our study is the first to carry out a series of experiments that involves related themes of growth, feeding, and reproduction of reef fish cycle as a potential tool to contribute to restoring Caribbean coral reefs. In Caribbean coral reefs, there is no reviewed literature about restocking experience (Obolski et al., 2016). Our results highlight the need to take advantage of the colonization phase when the postlarvae transform into juveniles and suffer catastrophic mortality rates (>90%) in the

week following colonization (Planes et al., 2002; Doherty et al., 2004).

The release stage for future implementation also requires several considerations. As a first step, we addressed the recording of the standard length and total length of individuals on the day of capture and the day of release. This was done to eliminate differences in the sizes of the juveniles released, as several studies indicate that larger individuals may have greater experience with predators, which may result in causing a wary behavior during the experiment (Rankin, 2010). While the pilot experiment with *S. partitus* showed high survival, damselfishes need to be tested in the context of coral restoration (Heenan et al., 2009). It is crucial to determine if they support or undermine the restoration efforts (Ladd et al., 2018) by algal farming which can lead to tissue mortality (Precht et al., 2010) and/or reduce the presence of other corallivorous by defending their territory (Schopmeyer and Lirman, 2015). It is reasonable to expect that the release of species with different behavioral and ecological characteristics can influence survivorship and migration rates, even in the processes of trophic interactions in coral reefs (Ladd and Andrew, 2020). However, in the context of the experimental setup species with similar life history and behavior (e.g., site fidelity) to that of other damselfishes, may be the starting point for scaling up to other species or groups.

Caribbean reefs have experienced unprecedented declines; they are characterized by coral-algal phase shifts in which coral cover is declining to be replaced by algae (Arias-González et al., 2017). The choice of release habitat should be considered using landscape analysis. For example, based on the relationship between habitat condition and target species, where habitats dominated by macroalgae or algal turfs can be supplied by herbivorous species such as grazers (surgeonfish), scrapers (parrotfish), and browsers (chubs) to provide a top-down control of algae (Green and Bellwood, 2009; Obolski et al., 2016). Caribbean reefs also have been changing in coral composition. Species of genus *Agaricia* spp. and *Porites* spp. tend to dominate the seascape (Perry et al., 2018). This scenario (Cozumel—Mexico) can be an opportunity for experiments to test diverse ecological functions.

Another characteristic habitat in the Mexican Caribbean region is the largely acroporid-dominated coral nurseries such as the current *ex situ* restoration sites of Wave of Change (Cozumel and Riviera Maya). This habitat has the recurrent prevalence of predators such as fireworm *Hermodice carunculata* that have a highly negative impact on populations of *A. cervicornis* and *A. palmata* (Calle-Triviño et al., 2017). These reefscapes can be benefited by the supply of fish such as white grunts (*Haemulon plumierii*) and sand tilefish (*Malacanthus plumieri*) (Ladd and Shantz, 2016). Coral growth can be improved by adding fishes such as grunts (*Haemulidae*) around coral nurseries or outplanting sites *via* delivery of fish-derived nutrients (Shantz et al., 2015) or *via* concentrate grazing by *Sparisoma* sp. and *Acathurus* sp. (Shantz et al., 2017; Calle-Triviño et al., 2021). Despite, the highest contributions of grunts and herbivorous fishes (surgeonfishes and parrotfishes) in both study sites our results suggest that there is a good chance of using these species to enhance research and may be considered in the context of restoration both structurally and functionally.

The primary method employed here can be used to broaden and predict the taxonomic composition and distribution of postlarval fishes. Also, it can promote the capture, culture, and release of reef fish with a sustainable approach, especially in enforcement and management sites across the Caribbean region. The results obtained are very promising in terms of species richness, diversity, abundance, and CPUE as well as an innovative way to drive restoration of coral reef services and functions. This sampling method provides the benefit of increasing the productivity of target species, for example, commercial and herbivorous fishes (Bell et al., 2009). These efforts can contribute to identifying settlement areas for reef fish, biodiversity monitoring (McLeod and Costello, 2017), managing of MPAs (Obolski et al., 2016), and supporting fisheries control through research and social engagement (Hein et al., 2020; Cortés-Useche et al., 2021). They can also support the restoration efforts of reefs that have suffered a loss in the resilience of their fish biodiversity (Lorenzen et al., 2010) or re-establish the provisioning services delivered by reefs in providing habitat and nursery areas for commercially (Hein et al., 2020) and functionally important species. In this sense, future work could be focused to improve sampling biodiversity and broadening monitoring variables that have an influence on recruitment such as luminosity, pH, algal blooms, wave exposure, rugosity, etc.

The pilot test (*S. partitus)* showed very promising results on survivorship relative to the settlement stage *ex situ.* This method tested here can be set up for a variety of fish species and seems to be a feasible restoration tool, to increase the benefits of management through the effective implementation that includes long-term ecological and economic synergies (Lirman and Schopmeyer, 2016; Cortés-Useche et al., 2019). Key aspects, such as fisheries policy and water quality treatment in the context of climate change and managing the connectivity of the tropical coastal reefscapes should be considered (Arias-González et al., 2016; Hein et al., 2020; Schmidt-Roach et al., 2020; Cortés-Useche et al., 2021).

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 authors.

ETHICS STATEMENT

The animal study was reviewed and approved by The Secretary of the Environment and Natural Resources approved field collection (PPF/DGOPA-282, DGOPA-DAPA-11811).

AUTHOR CONTRIBUTIONS

CC-U, JA-G, JC-P, and WR-G conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the

paper, and approved the final draft. RY and JC-T analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft. AC-F conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft. RR-F performed the experiments, authored or reviewed drafts of the paper, and approved the final draft. All authors contributed to the article and approved the submitted version.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.718526/full#supplementary-material

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Increased Coral Larval Supply Enhances Recruitment for Coral and Fish Habitat Restoration

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Loss of foundation reef-corals is eroding the viability of reef communities and ecosystem function in many regions globally. Coral populations are naturally resilient but when breeding corals decline, larval supply becomes limiting and natural recruitment is insufficient for maintaining or restoring depleted populations. Passive management approaches are important but in some regions they are proving inadequate for protecting reefs, therefore active additional intervention and effective coral restoration techniques are needed. Coral spawning events produce trillions of embryos that can be used for mass larval rearing and settlement on degraded but recoverable reef areas. We supplied 4.6 million *Acropora tenuis* larvae contained in fine mesh enclosures *in situ* on three degraded reef plots in the northwestern Philippines during a five day settlement period to initiate restoration. Initial mean larval settlement was very high (210.2 ± 86.4 spat per tile) on natural coral skeleton settlement tiles in the larval-enhanced plots, whereas no larvae settled on tiles in control plots. High mortality occurred during early post-settlement life stages as expected, however, juvenile coral survivorship stabilised once colonies had grown into visible-sized recruits on the reef by 10 months. Most recruits survived and grew rapidly, resulting in significantly increased rates of coral recruitment and density in larval-enhanced plots. After two years growth, mean colony size reached 11.1 ± 0.61 cm mean diameter, and colonies larger than 13 cm mean diameter were gravid and spawned, the fastest growth to reproductive size recorded for broadcast spawning corals. After three years, mean colony size reached 17 ± 1.7 cm mean diameter, with a mean density of 5.7 ± 1.25 colonies per m^{-2}, and most colonies were sexually reproductive. Coral cover increased significantly in larval plots compared with control plots, primarily from *A. tenuis* recruitment and growth. Total production cost for each of the 220 colonies within the restored breeding population after three years was United States $17.80 per colony. A small but significant increase in fish abundance occurred in larval plots in 2018, with higher abundance of pomacentrids and corallivore chaetodontids coinciding with growth of *A. tenuis* colonies. In addition, innovative techniques for capturing coral spawn slicks and larval culture in pools *in situ* were successfully developed that can be scaled-up for mass production of

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larvae on reefs in future. These results confirm that enhancing larval supply significantly increases settlement and coral recruitment on reefs, enabling rapid re-establishment of breeding coral populations and enhancing fish abundance, even on degraded reef areas.

Keywords: reef restoration, sexual reproduction, *Acropora tenuis*, larval settlement, coral recruitment, coral growth, survivorship, fish assemblages

INTRODUCTION

Scleractinian hermatypic corals are foundation species on coral reefs (Bruno and Selig, 2007; Harrison and Booth, 2007), and function as ecosystem engineers with essential roles in calcification and reef accretion, creating crucial three-dimensional habitats for many other reef organisms (Birkeland, 1997; Burke et al., 2011; Hoegh-Guldberg et al., 2017). Healthy coral populations consist of highly fecund colonies that produce large numbers of gametes for broadcast spawning and planktonic larval development, or cyclic production of brooded planulae (Harrison, 2011; Randall et al., 2020). As with other marine invertebrates, the efficiency with which coral larval production results in successful settlement, survival, recruitment and growth into adult breeding colonies is unknown (Harrison and Wallace, 1990). Marine invertebrates have high intrinsic mortality, with losses from predation during their planktonic phase estimated to be up to 90–100% daily (Thorson, 1950; Rumrill, 1990; Pechenik, 1999), and pelagic larval dispersal results in many coral larvae being carried away from reefs in currents (Harrison and Wallace, 1990; Jones et al., 2009). Therefore, less than 0.1–0.001% of progeny may settle and survive to adult size on coral reefs. However, the low rate of larval settlement and recruitment is offset by production of vast quantities of coral spawn and billions or trillions of larvae, resulting in sufficient recruitment to maintain coral populations and enable recovery from most natural disturbances over decadal timescales (Connell et al., 1997; Gilmour et al., 2013; Gouezo et al., 2019).

Coral communities in many reef regions have been decimated by increasing anthropogenic disturbances including overfishing and destructive blast fishing (McManus et al., 1997; Wilkinson, 2008), coastal development, pollution and increasing predator and disease outbreaks (Bruno and Selig, 2007; Burke et al., 2011), which are exacerbated by climate change induced increased severity and frequency of marine heatwaves and mass coral bleaching (Hoegh-Guldberg et al., 2017; Hughes et al., 2018) and extreme storm events (De'ath et al., 2012; Jackson et al., 2014). The loss of large numbers of breeding corals significantly reduces gamete production, fertilisation rates and larval production (Harrison and Wallace, 1990; Randall et al., 2020), thereby impairing or preventing sufficient larval recruitment for natural recovery of coral populations and communities (Hughes et al., 2019). Reduced larval supply limits recruitment and accelerates decline in coral communities, creating opportunities for other reef invertebrates and algae to colonise, and can contribute to phase shifts from coral to algal dominated reef systems (Done, 1992; Bruno et al., 2009).

Passive reef management approaches are proving to be ineffective in enabling coral and reef communities to recover from chronic anthropogenic and natural disturbances in many coral reef regions around the world (Burke et al., 2011). Therefore, increasing attention and research has focussed on active coral restoration. Coral restoration can use either asexual fragmentation and cloning methods, or sexual production of corals to promote recovery, but current scales of restoration are limited (reviewed by Rinkevich, 1995; Edwards, 2010; Omori, 2019; Boström-Einarsson et al., 2020).

Asexual fragmentation and nursery outplanting methods can quickly increase clonal corals on high-value reef patches, but are inherently limited by high production costs and ongoing maintenance costs for managing nurseries (Edwards, 2010; Omori, 2019). Most early coral restoration projects relied on asexual coral fragmentation and transplantation to attempt to re-establish corals on degraded reef areas (Rinkevich, 1995; Edwards, 2010). Although conceptually simple, coral fragmentation and outplanting is often limited in scale, can be costly, and clonal fragments have limited genotypic diversity that reduces adaptive capacity and resilience of transplanted populations (Baums, 2008; Boström-Einarsson et al., 2020). More recent advances include the use of nurseries for enabling recovery of coral fragments before outplanting ("coral gardening"), which can lead to higher rates of survival and growth, but requires higher maintenance that increases production costs (Lirman and Schopmeyer, 2016; Rinkevich, 2019; Boström-Einarsson et al., 2020).

Sexual reproduction and mass culture of larvae enables enhanced genetic diversity and evolutionary potential among offspring derived from more tolerant surviving adult broodstock corals, and is potentially scalable to larger reef areas. However, broadcast spawning corals and other marine invertebrates with similar life histories have inherently high rates of mortality post-settlement due to strong environmental selective pressures operating on these invisible early stages of coral reproduction (Harrison and Wallace, 1990; Wilson and Harrison, 2005; Doropoulos et al., 2016; Randall et al., 2020).

Various forms of sexual propagation have been developed to overcome the inherent genetic limitations of asexual propagation for restoration programmes. These include settlement of cultured coral larvae on artificial substrata, and nursery rearing before transplantation and deployment on reefs (Omori, 2005; Petersen et al., 2005; Baria et al., 2012; Guest et al., 2014; Chamberland et al., 2017). Nursery rearing can sometimes increase survival rates of outplanted juvenile corals but also increases the production costs (Guest et al., 2014). Larval settlement in laboratory environments may also create artificial selection

pressures and fitness consequences that result in *ex situ* settled juveniles being maladapted to environmental conditions on reefs when transplanted, leading to suboptimal survival and growth rates.

An alternative approach is larval enhancement whereby larvae are settled directly onto reef areas or artificial surfaces *in situ*, which increases the chances of successful settlement in preferred microhabitats and improved genotype-environment matching (Harrison et al., 2016; Harrison, 2021). However, relatively few studies have used this approach, and the initial studies were done on healthy reefs with naturally high larval supply and recruitment (Heyward et al., 2002; Suzuki et al., 2012; Edwards et al., 2015). Additionally, monitoring of settlers' survival, growth and recruitment was performed for periods of six weeks (Heyward et al., 2002), six months (Suzuki et al., 2012) and approximately 13 months (Edwards et al., 2015). Therefore, these studies were unable to determine the longer-term restoration outcomes of the approach.

In 2013, dela Cruz and Harrison (2017) supplied ∼400,000 *Acropora tenuis* larvae into replicate 24 m^2 reef plots on degraded reef areas in the northwestern Philippines and recorded high rates of initial larval settlement on natural settlement tiles in plots supplied with larvae and no settlement in control plots. Survival and growth of settled spat and recruits were monitored for three years, with significantly higher recruitment on larval enhancement plots compared with control plots. Larval enhancement resulted in an average of 2.3 colonies m^{-2} surviving on available reef substrata after three years, and re-established a breeding population on this degraded algal phase-shifted reef (dela Cruz and Harrison, 2017).

The present study builds on the successful pilot study of dela Cruz and Harrison (2017) and aimed to (1) quantify the effects of increased *A. tenuis* larval supply on initial larval settlement and recruitment rates on degraded reef substrata, and monitoring survival and growth of colonies in the restored population through to reproductive size, (2) assess longer-term restoration outcomes of larval enhancement on changes in coral and other benthic communities and fish assemblages over three years, and (3) develop new techniques for capturing larger volumes of coral spawn directly on the reef, for future larger-scale restoration.

METHODS

Location and Experimental Design

Degraded reef areas at Magsaysay reef (16°31′36″ N, 120°02′01″ E) in the Magsaysay Marine Protected Area (MPA) in Northern Luzon, Philippines were used for this study. Magsaysay reef is part of the Bolinao-Anda Reef Complex (BARC) in the Lingayen Gulf located in the municipality of Anda, Pangasinan (**Figure 1**). This experiment sought to quantify the effects of supplying high densities of coral larvae onto replicate degraded reef plots during their settlement period, then monitoring initial larval settlement rates, subsequent survival and growth of recruits and adult corals over three years. Repeated monitoring compared the effects of larval restoration on benthic and fish assemblages, particularly fish abundance, species richness and trophic groups, between larval enhancement and control plots where no cultured larvae were supplied.

Site Selection and Settlement Tiles

Prior to the experiment in April–May 2016, six replicate 5 × 5 m plots were haphazardly selected on degraded reef areas at 3–4 m depth on Magsaysay reef. Plots were located at least 10 m apart, with three plots haphazardly assigned as larval enhancement plots and three plots as controls that did not receive larvae.

Newly settled coral polyps (spat) are microscopic and cryptic, hence are very difficult to detect on complex natural reef substrata (Harrison and Wallace, 1990). Therefore, initial rates of larval settlement were quantified using biologically conditioned 10 × 10 cm by 3–4 cm thick settlement tiles cut from dead tabulate *Acropora* skeletons collected from the intertidal zone beside Cory reef, near Magsaysay reef (**Figure 1**). Each tile was identified with a coded metal tag and biologically conditioned for four weeks in aerated flow-through seawater tanks at the Bolinao Marine Laboratory (BML) of The Marine Science Institute, University of the Philippines, prior to use on the reef plots.

Just prior to the experiment, tiles were examined to confirm that no coral recruits were present. Then ten tiles were deployed haphazardly in each of the three larval enhancement plots and in each of the three control plots, with each tile located on a separate small stainless steel post on a baseplate attached into the reef (after Mundy, 2000). A small 0.5–1 cm gap was left between each tile and its baseplate to allow larvae to settle on all tile surfaces. In addition, a total of 12 open reef control settlement tiles were also haphazardly deployed on posts near the corners of control plots (one open reef control tile at each corner of each of the three control plots), to monitor natural coral larval settlement patterns during the larval settlement period when the experimental plots were covered with fine mesh enclosures. The surface areas of the natural coral skeleton tiles were estimated to be about 360 ± 3.7 cm^2 (SE) by 3D scanning thirty representative tiles (dela Cruz and Harrison, 2017). Minimal variation in surface areas among tiles allowed settlement rates to be standardised between tiles.

Acropora tenuis Spawning and Larval Culture

Four days prior to the full moon on 22 April 2016, colonies of *Acropora tenuis* were examined *in situ* on Magsaysay reef and Caniogan reef (16°30′26.8″ N, 120°0′47.7″ E; **Figure 1**) by carefully breaking a few branches to determine if colonies contained pink to red coloured mature eggs, indicating imminent spawning (Harrison et al., 1984). Twenty-three gravid colonies from Magsaysay reef and six gravid colonies from Caniogan reef were collected from ∼2–4 m depths and transferred to the BML aquaculture facility and maintained in an apparently healthy condition in large tanks with flow-through seawater and aeration. Coral colonies were monitored from ∼6 pm each evening during the crepuscular period to check for setting and spawning behaviours, and spawning of all 29 colonies occurred between 1830 and 1900 h on 23 April 2016 (1 night after full moon, nAFM, **Table 1**) in the BML tanks.

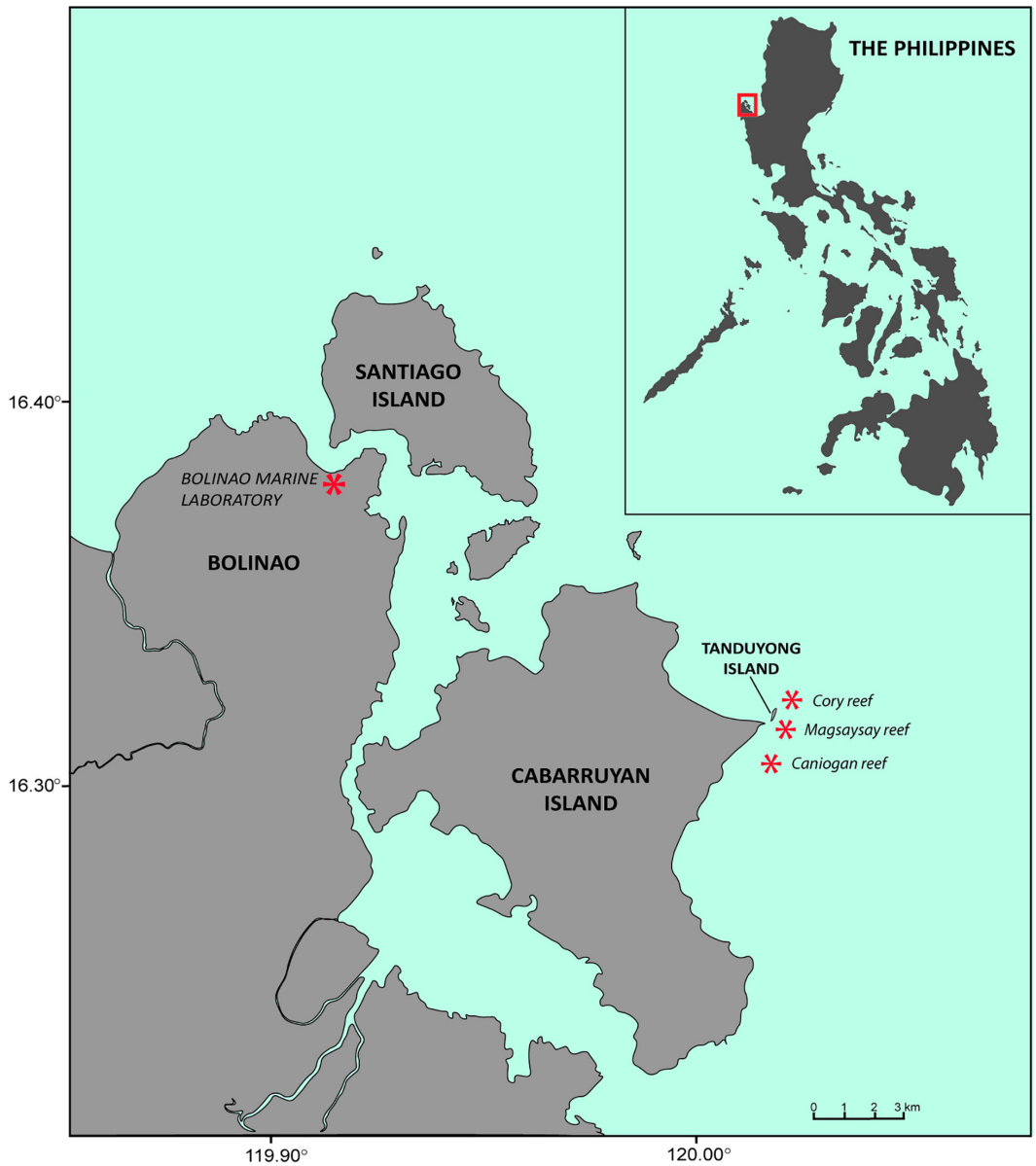

FIGURE 1 | Location of coral restoration site at Magsaysay reef and Bolinao Marine Laboratory in Northern Luzon, Philippines.

In addition, gravid three-year old *A. tenuis* F1 colonies grown from larvae that settled in larval restoration plots at Magsaysay reef in 2013 (dela Cruz and Harrison, 2017), were monitored during night dives to enable collection of spawned gametes from their first gametogenic cycle. At ∼1730 h each evening, spawn collection cones were carefully placed over gravid colonies until

TABLE 1 | Lunar periodicity of *A. tenuis* spawning in 2013 and 2016 studies.

Year	*A. tenuis* population	Location of spawning	nAFM	Notes
2013	Collected wild colonies	BML	3–4	dela Cruz and Harrison (2017)
2016	Collected wild colonies	BML	1	Present study, dela Cruz and Harrison (2017)
	Reproductive F1 colonies from 2013 study	*In situ*	2	
2018	Reproductive F2 colonies from 2016 settlement	*In situ*	1–2	Present study
2019	Reproductive F2 colonies from 2016 settlement	*In situ*	4–5	Present study

spawning occurred, then removed at ∼1930 h if spawning did not occur. The 40 cm diameter spawn collection cones were made from fine 180 μm organza cloth attached to a weighted metal ring, with the upper section connected to an inverted 800 mL clear plastic collection jar partially filled with air to keep the conical net floating upright. The first spawning of these three-year old restoration colonies occurred between 1830 and 1900 h on 24 April 2016 (2 nAFM, **Table 1**), and egg-sperm bundles were collected from 31 colonies. The plastic jars containing gametes were transported to the BML aquaculture facility and mixed together immediately in a 50 L container.

Millions of gametes from the spawned egg-sperm bundles were collected from the 29 collected colonies (*ex situ*) and the 31 three-year old F1 colonies (*in situ*), and each cohort was cultured separately in the BML aquaculture facility using standard methods as follows. Gamete bundles from all colonies that spawned together were collected and transferred into a fertilisation tank containing 20 L of 1 μm filtered seawater and gently mixed to facilitate cross-fertilisation (Harrison, 2006; dela Cruz and Harrison, 2017). After a fertilisation period of 1–1.5 h to optimise cross-fertilisation (dela Cruz and Harrison, 2020a), excess sperm was removed from the tank by siphoning water from beneath the floating eggs and embryos to prevent polyspermy and degraded water quality (Willis et al., 1997). The seawater with sperm was slowly replaced with new 1 μm filtered seawater, and the sperm washing process was repeated three times. After an hour, subsamples of eggs and embryos were removed and examined under a stereomicroscope to quantify percentage fertilisation.

Developing embryos were skimmed off the water surface and transferred to large rearing tanks (> 1,000 L and 500 L) at densities of 4–5 embryos cm^{-2} and gently agitated. After 24 h development when embryos had formed into more robust spheroidal planula larvae (Harrison and Wallace, 1990), gentle aeration was supplied and > 100 L of seawater was syphoned from the bottom of each tank and replaced with 1 μm filtered seawater each day to maintain water quality and healthy larval cultures. Larvae were cultured until they became competent to settle and were used for settlement trials.

Confirming Larval Competence

On 29 April when F2 larval cohorts were 4.5 days old (from F1 corals that spawned *in situ* on the reef) and larvae from colonies that spawned *ex situ* at BML were 5.5 days old, samples of larvae were carefully filtered from culture tanks using fine plankton mesh sieves and counted under stereomicroscopes illuminated with fibre-optic and LED lights into five subsamples of 100 larvae from each cohort. Larvae were transferred in plastic jars to Magsaysay reef where each sample was placed inside a small plastic settlement cage with plankton mesh sides (after Ward and Harrison, 1997), with each settlement cage containing two biologically conditioned settlement tiles. These initial settlement trials showed that 12.4% ± 1.8 of the wild larvae and 18.4% ± 4.8 of the F2 cohort larvae settled within 5 days, confirming that larvae were healthy and competent to settle.

On 1 May, after seven days culture for the F2 cohort and eight days culture for the *ex situ* spawning cohort, competent swimming larvae were concentrated using fine plankton mesh sieves and combined into separate 160 L holding tanks for each cohort. Larvae were thoroughly mixed, then three 60 mL subsamples were taken from each tank and counted under stereomicroscopes to estimate total larval abundance in each cohort. A total of 4.6 million larvae were available from the cultures for the reef settlement experiment. These larvae were thoroughly mixed again to homogenise their distribution and then evenly distributed into fifteen 20 L plastic bags supplied with oxygen and sealed for transport to Magsaysay reef for the larval enhancement experiment (after dela Cruz and Harrison, 2017).

Coral Larval Enhancement

To retain larvae on the three larval enhancement plots during the settlement period, each treatment plot was enclosed in a purpose-designed 5 × 5 m by 60 cm deep square tent-like enclosure constructed from 180 μm plankton mesh net (**Figure 2A**). The net was reinforced with webbing sewn along the seams, and 50 × 50 cm larval supply portals made from vinyl with velcro sealing were located in each quadrant on the upper surface (**Figure 2B**). A PVC pipe frame around the perimeter and across the centre of the plot supported the integrity of the net shape. The net was secured onto the frame with webbing ties sewn at 1 m intervals along the perimeter, at each corner and in the centre. Steel reinforcing bars were driven into the reef at each corner and at intervals along the sides. Net sides were bordered with a 20 cm vinyl collar with a 10 mm rope sealed along the edge for increased strength. Regularly spaced 10 mm holes in the vinyl above the sealed rope allowed metal pegs to secure the base of the net to the seafloor. Additional concrete bricks and dead coral skeletons were placed along the vinyl collar to help seal the base of the net onto the reef. A small buoy was attached to the centre of the net to keep the upper surface of the net slightly above the reef to reduce abrasion of the net on corals and reef substrata (**Figure 2A**). The bottom of each mesh tent enclosure was therefore open to the reef benthic environment within each plot to enable larvae to settle on suitable dead coral substrata.

An estimated 1.54 million *A. tenuis* larvae were added into each of the three 5 × 5 m larval mesh nets on the larval enhancement plots by divers sequentially opening each of the larval portals and transferring the larvae in five plastic bags into the net (**Figure 2B**). The portal was then partly closed and a diver wafted the larvae further into the enclosure using a dive fin to increase the spread of larvae across the plot, then the portal was firmly resealed to prevent larvae drifting out of the net. Control plots were also covered with nets during the settlement period but no larvae were added.

Monitoring Settlement

On 5 May 2016 after four days larval settlement, strong winds and wave action were impacting the Magsaysay reef sites and the base of the larval mesh net in larval plot 3 had partly detached, so this net was removed to avoid damage to corals and the reef. On 6 May, the larval mesh enclosures were removed from the remaining two larval plots and the three control plots. The 60 settlement tiles from the six experimental plots (ten tiles in each of the three larval enhancement plots and in each of

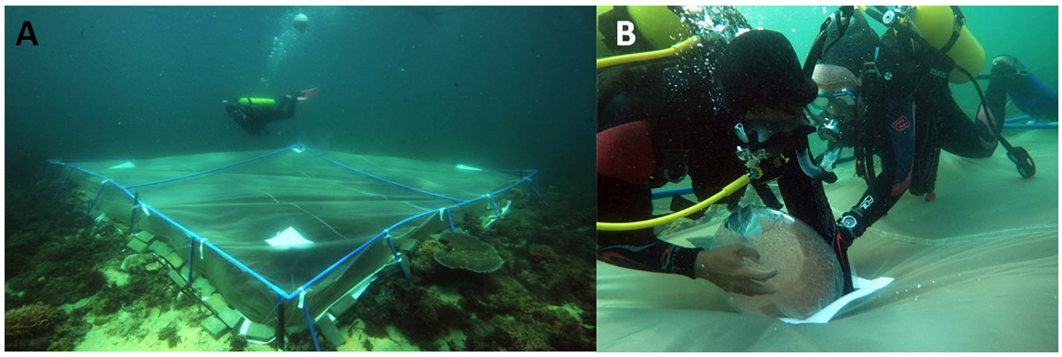

FIGURE 2 | Larval mesh tent enclosure with frame attached onto reef **(A)**, and divers adding concentrated *A. tenuis* larvae into a re-sealable portal on the mesh enclosure for settlement **(B)**.

the three control plots) and the additional 12 settlement tiles deployed around the outside of control plots as open reef controls were carefully collected and transferred in seawater to a nearby temporary field station on Tanduyong Island (**Figure 1**). Tile surfaces were viewed while submerged in small trays under stereomicroscopes illuminated with fibre-optic and LED lights, and numbers of settled coral spat and their locations on tiles were recorded. After monitoring, tiles were returned to their specific locations on the reef plots and reattached onto the tile posts with the correct orientation, to enable repeated monitoring of growth and survival of spat on the tiles.

Monitoring Survival, Growth and Visible Recruitment

Initial survival on tiles was monitored after 1 and 2 months post-settlement by collecting the tiles and counting the surviving spat on each tile surface under stereomicroscopes at Tanduyong Island. Tiles were subsequently returned to the reef and reattached to their numbered tile posts. After 10 months, recruits on tiles had grown large enough to census underwater, so survival and growth were monitored *in situ* at 10, 12, 14, 17, 20, 25, 27, 29, 32, and 34 months post-settlement. The *A. tenuis* recruits that had settled on the reef surfaces within each of the larval enhancement plots were also visible underwater by 10 months, and their characteristic morphology and subsequent growth into recognisable *A. tenuis* colonies were used to identify them as originating from larvae that settled on the reef during the larval enhancement experiment. Each recruit and juvenile colony on the reef were mapped and a small, numbered metal tag was permanently attached nearby to facilitate monitoring at the same time as recruits on tiles were monitored.

Colony size was measured using vernier callipers to measure the length (l), width (w) and height (h) of each *A. tenuis* coral on the recruitment tiles and on the natural reef substrata. The mean planar diameter was calculated from the maximum and minimum diameters measured for each colony. The approximate volume was also measured following a spherical formula $EV = \pi r^2 h$, where $r = (l + w)/4$ (Shaish et al., 2010). In some cases, larvae settled in close proximity to others and colonies grew together, fusing into a larger chimeric colony. Individual colonies that fused together were still counted and measured separately when polyp demarcation or separation lines were still visible. Where individual corals were indistinct in a fused coral colony, the previous individual count was recorded for survivorship, but measured as a single fused colony for growth.

Sexual Reproduction of Recruits

Evidence of sexual reproduction in all surviving recruits was assessed at 23 and 34 months after settlement, just prior to potential spawning periods after full moons when corals were 2 and 3 years old. Coral reproductive status was examined by carefully breaking up to three small branches to observe whether developing and pigmented oocytes and spermaries were present in broken sections of polyps (after Harrison et al., 1984). Branches were then gently wedged back into the colony to avoid loss of tissues and spawning biomass.

Coral Production Costs

The costs of producing coral recruits including all materials, vessel hire and fuel, diving, labour, larval rearing, and capital costs for larval mesh tents were estimated following Edwards (2010). The average costs per colony were estimated by dividing the total costs by the total numbers of recruits alive at 10 months and at 3 years in the larval plots. Costs were initially calculated in Philippine Peso (PhP) and Australian Dollar (AUD), and converted to United States Dollar (USD) values.

Assessing Reef Community Status

Prior to the early 1980s, BARC reefs were characterised by relatively high 30–50% mean live coral cover and healthy reef status (Gomez et al., 1981). However, extensive blast fishing, aquaculture development and crown-of-thorns starfish outbreaks severely impacted coral and other reef communities in the Lingayen Gulf, resulting in degradation of these reefs during subsequent decades (Cruz-Trinidad et al., 2009; dela Cruz and Harrison, 2017). Blast fishing has since been banned and has now effectively ceased in local communities from Anda and nearby towns, hence these reefs are now potentially recoverable but are limited by low rates of natural coral recruitment (dela Cruz and Harrison, 2017).

Reef Benthic Community

To quantify benthic cover of corals, other benthos and the reef community status prior to larval enhancement, digital images of the experimental plots were taken in April 2016 using a Canon G1-X underwater camera and a 1 × 1 m quadrat frame, and images were analysed using CoralNet (Beijbom et al., 2015). Twenty-five random sampling points were generated for each image and the benthic category underlying each point was identified, resulting in 625 points per plot. Only one adult *A. tenuis* colony was present inside the reef plots prior to the experiment, growing in control plot 2. Some excess macroalgae within the plots was carefully removed by divers to reduce potential physical and allelopathic effects of algal biomass on larval settlement (Ceccarelli et al., 2018). Monitoring of the reef benthic community status was repeated in March 2019 to quantify changes over time in the experimental plots.

Reef Fish Assemblage

Modified Stationary Point-Counts (Bohnsack and Bannerot, 1986) were used to survey the reef fish assemblages in all plots. Ten-minute surveys were completed in each plot, and all non-cryptic fishes inside the plots were identified to species and recorded (Allen et al., 2005), and the total length of individual fishes was visually estimated (in cm). Monitoring of the reef fish assemblages in all plots was repeated in March 2017, 2018, and 2019 to quantify changes.

In situ Multispecific Coral Spawn Slick Collection and Larval Rearing

A further objective of this study was to develop new techniques for capturing large volumes of floating coral spawn slicks and culturing embryos and larvae in floating mesh net enclosures at sea for future similar experiments. Millions of spawned egg-sperm bundles were collected *in situ* on Magsaysay reef from large multispecific spawning events on 31 March 2016 (8 nAFM) and 1 April 2016 (9nAFM) and placed in a mesh rearing pool suspended from a 5 × 5 m floating bamboo frame. The rearing pool was 5 × 5 m square by 3.5 m deep with the upper part of the net system comprising a vinyl sheet extending from 0.5 m above the sea surface to 0.5 m below the surface (**Figure 3A**). All net systems for larval rearing or spawn slick collection (described below) feature vinyl sheeting above and below the waterline, as this smooth surface avoids abrasion of the delicate developing embryos on the plankton mesh. The lower portion of the larval rearing pool net comprised 180 μm plankton mesh net that extended down to the reef substratum where it was temporarily attached using small steel bars and pegs (**Figure 3B**). The net was located over a small patch of healthy reef dominated by large gravid *Acropora hyacinthus* and *A. cytherea* colonies, so that spawned egg-sperm bundles would float up and be retained within the net. Additional coral spawn was collected using coral spawn collection cones placed over other spawning *Acropora* spp. colonies observed on night dives, and by swimming spawn cones along the sea surface as neuston nets to collect samples of large spawn slicks that were then added into the larval rearing pool. Samples of developing embryos were taken 12 and 20 h after spawning to quantify development stages and health of the cultures.

The spawn slick capture and larval culture pools concept was further developed by designing an innovative floating "spawn slick capture" net with two 15 m long booms that extended each side of a partly submerged net to funnel buoyant coral spawn slicks into the collector. A 2 × 2 m PVC pipe prototype semi-submersible frame was built in April 2016 and the design was further refined in 2017 during field trials on the reef. In March 2017, a 150 μm plankton mesh net with an upper vinyl panel extending 0.5 m above and below the sea surface, and paired 15 m inflatable spawn collector booms were attached to a 5 × 5 m floating bamboo frame which was positioned into the wind and down current from spawning corals on Magsaysay reef (**Figure 3C**). The booms were held apart at ∼90° using anchor ropes, and had a 30 cm weighted vinyl curtain submerged below the water surface to facilitate spawn slick collection (**Figure 3C**). Subsequently, the submerged 150 μm larval culture mesh nets were redesigned to be free-floating within a frame, and with tapered sides and a zipper opening at the base. These redesigned nets were attached to stronger 5 × 5 m floating steel frames to increase the success of spawn collection and larval culture on the reef in adverse weather conditions (**Figure 3D**). Large multispecific *Acropora* spp. spawning events occurred at Magsaysay reef on 20 and 21 March 2017 (8–9nAFM) and on the 12 and 13 March 2018 (10–11nAFM, **Table 1**), and spawned egg-sperm bundles were collected in the spawn catchers, and larvae were reared in the larval culture nets attached to the floating frames. In 2018, a solar powered seawater pump and aeration system was attached to the floating steel frames to maintain good water quality and increase the efficiency of larval cultures on the reef.

Environmental Monitoring

Environmental conditions on the Magsaysay reef site were monitored periodically throughout the study from January 2016 to March 2019. Sea temperatures were monitored using Stowaway temperature data loggers deployed at 3 m depth near the experimental plots periodically from January 2017 to April 2018. Additional sea temperature, salinity, dissolved oxygen (DO), and pH water quality parameters were monitored during field trips using a portable Horiba multiprobe instrument at depths of 3–4 m. Additional sea surface temperature (SST) data from January 2016 to March 2019 were obtained from coralreefwatch@noaa.gov (**Supplementary Figure 1**), and light intensity was measured using a LI-COR®193SA spherical quantum sensor attached to a LI-COR® LI-1400 data logger (**Supplementary Figure 2**). The ten to fifteen consecutive light readings were obtained at noon during monitoring field trips, and values averaged.

Data Analyses

Coral Larval Enhancement

The three larval enhancement plots and the three control plots were used as statistical replicates ($N = 3$). Data from the groups of ten tiles within each plot were averaged to quantify mean initial settlement rates, and subsequent growth and number of

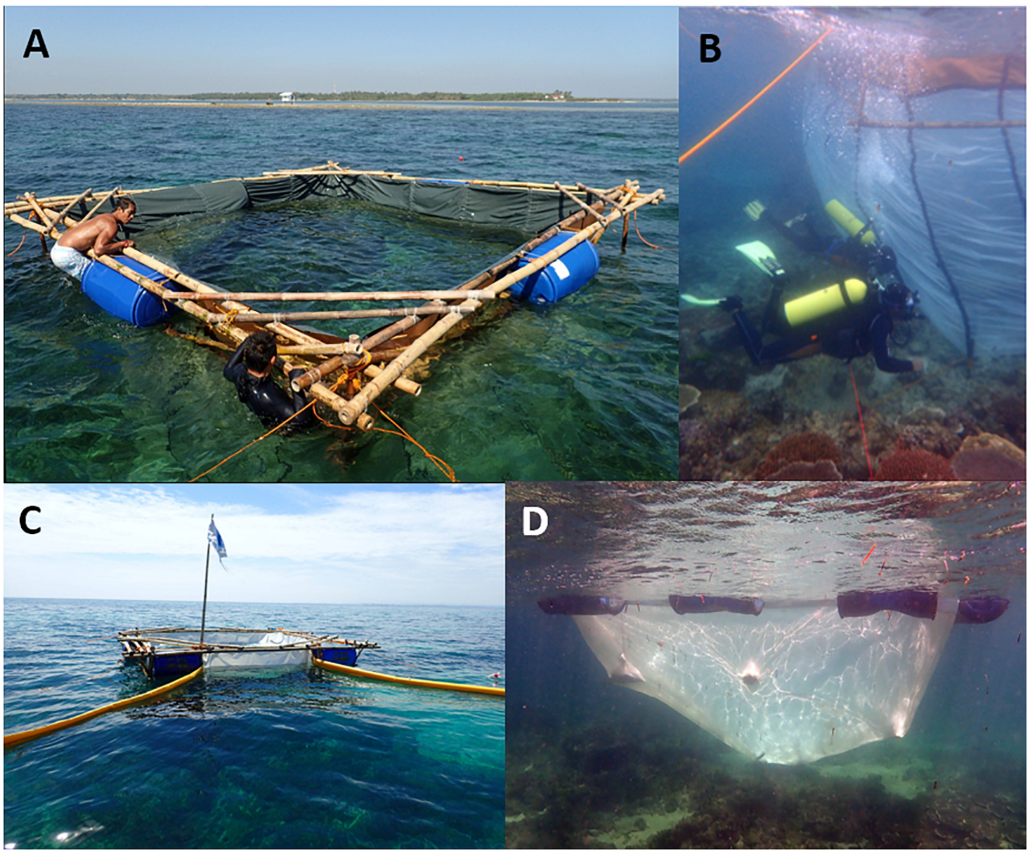

FIGURE 3 | A prototype 5 × 5 m floating bamboo frame larval rearing pool deployed on Magsaysay reef in 2016 **(A)**, and the submerged mesh curtain attached to the bamboo frame on reef site **(B)**. Spawn catcher with booms attached to bamboo frame in 2017 on Magsaysay reef **(C)**, and 2018 larval rearing net deployed under a floating steel frame **(D)**.

surviving recruits at each monitoring period up to age 34 months. Data are reported as mean values ± standard error. Differences in larval settlement patterns on different tile surfaces from the larval-enhanced plots after five days of larval settlement were tested using one-way ANOVA. Tukey's HSD test was conducted *post hoc* to determine any significant differences in settlement patterns among tile surfaces. The survivorship of coral recruits on different tile surfaces was analysed using non-parametric pairwise comparison survival tests, based on the Kaplan–Meier function (Lee and Wang, 2003). Significant differences in survival patterns of juvenile corals on natural substrata and on tiles from 10 to 34 months after larval settlement were also tested using the same analysis. Growth rates of juvenile corals on recruitment tiles versus growth rates on natural substrata were compared using one-way ANOVA, and significant increases in growth of juvenile corals through time were determined using repeated measures MANOVA. To determine if the assumptions of ANOVA were met, Shapiro-Wilk normality tests and Levene's test of homoscedasticity were used on each independent variable.

Benthic Communities

Benthic community patterns within and among experimental plots were compared both before larval enhancement and three years after the larval restoration, using analysis of similarities (ANOSIM) and PERMANOVA (using PRIMER v6) to test for similarities and significant differences in the per cent benthic cover composition of major benthic categories including live corals, soft corals, sponges, other invertebrates, macroalgae, dead coral covered with turf algae, sand, dead coral and rubble.

Reef Fish Assemblages

Differences in fish species richness and fish abundance between larval enhancement and control plots were analysed using Mann-Whitney *U*-Test in Statistica® software. Fish species composition between larval enhancement and control plots both before and three years after the larval restoration was graphically presented in two-dimensional ordination plots by non-metric multidimensional scaling (nMDS) using the Bray-Curtis measure of similarity (PRIMER v6). Data were transformed to fourth root so that each species contributed evenly to each analysis. Two-way ANOSIM (analysis of similarity) with pair-wise comparisons was conducted to formally test the significant differences between controls and each treatment. Similarity percentage procedure (SIMPER) was also employed to identify the fish species that contributed to the dissimilarities.

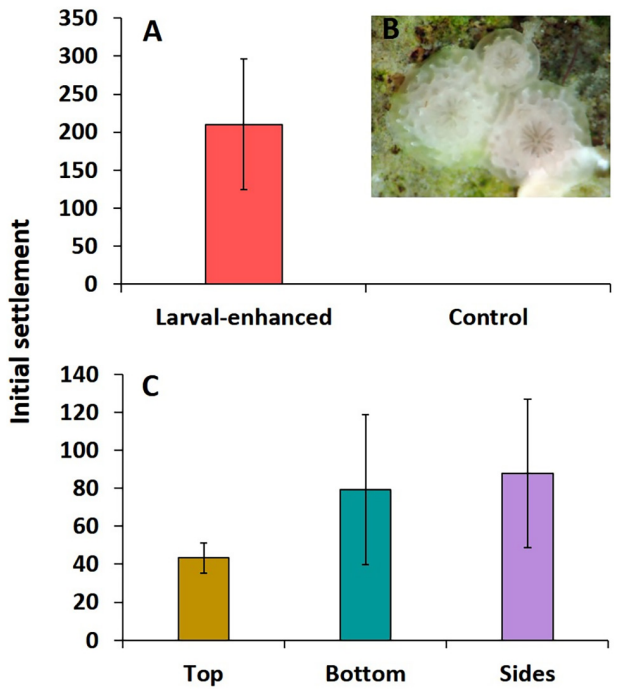

FIGURE 4 | Initial mean *A. tenuis* larval settlement on tiles in larval enhanced ($N = 3$) and control ($N = 3$) plots after 5 days larval settlement **(A)**, three newly settled spat with well developed primordial skeletons **(B)**, and mean settlement on different tile surfaces in the larval enhanced plots **(C)**. Error bars are ± SE.

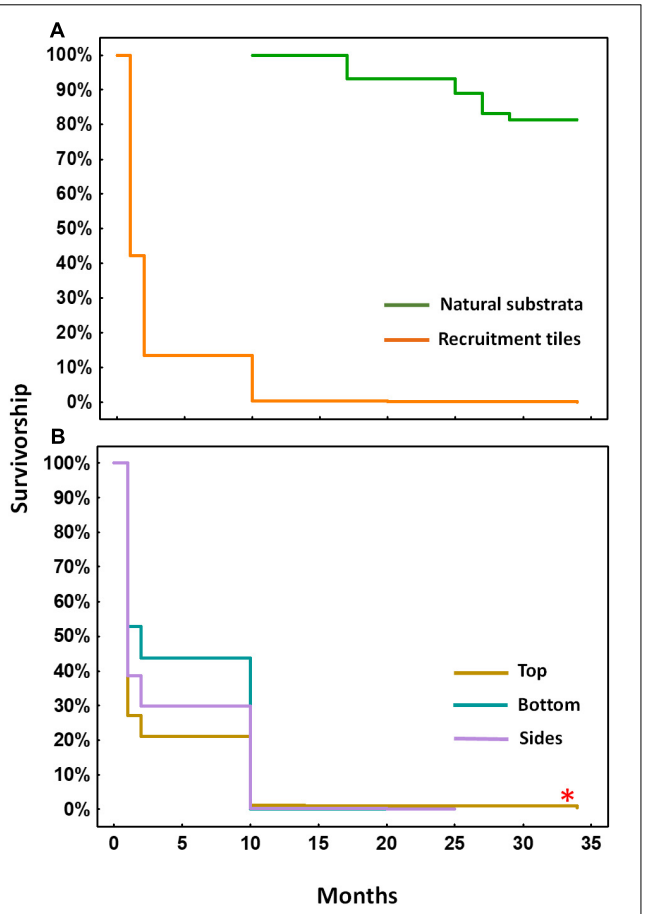

FIGURE 5 | Kaplan-Meier survivorship over 34 months for settled *A. tenuis* polyps, juveniles and recruits in **(A)** larval enhanced plots on tiles (orange) and for visible recruits on natural reef substrata (green) starting at 10 months after larval settlement, and **(B)** survivorship of recruits on different tile surfaces in the larval enhanced plots. Asterisk denotes significant difference between tile surfaces.

RESULTS

Coral Larval Enhancement

Larval Settlement Patterns

A total of 1,617,000 (± 124,910.20 SE) 8-day old larvae and 3,008,000 (± 244,767.50 SE) 7-day old F2 larvae were available from the cultures and these were divided equally into three groups for deployment in the three larval enhancement plots. High initial larval settlement rates were recorded on tiles in the three larval plots that had each been supplied with an estimated 1.54 million *A. tenuis* larvae. A total of 6,307 settled spat were recorded on the thirty tiles, with a mean of 210.2 ± 86.36 spat per tile (**Figure 4A**). Coral spat had well-developed primordial skeletons indicating rapid settlement after release onto the reef plots (**Figure 4B**). Mean larval settlement per tile was highest in larval plot 1 (382.2 ± 116.18), lower in plot 2 (138.2 ± 37), and lowest in plot 3 (110.3 ± 43). Highest mean settlement rates occurred on the sides and bottom surfaces of tiles with lower rates on the top surfaces, but these were not significantly different ($F = 0.53$, $P = 0.61$) (**Figure 4C**). No *A. tenuis* or other larvae settled on tiles in control plots covered in mesh enclosures (**Figure 4A**), and no larvae settled on the 12 tiles located around the outside of the mesh enclosures on the control plots, indicating there was no natural recruitment during the five-day larval settlement experiment.

Recruit Survivorship

As expected, repeated monitoring of settled spat on tiles showed a Type III survivorship pattern characterised by high rates of mortality during the first two months after settlement and lower mortality from two to ten months (**Figure 5A**). Recruits grew large enough (1.9 ± 0.26 cm mean diameter) to be visible on tiles and on reef substrata after ten months, enabling *in situ* monitoring of survival and growth. On the settlement tiles, a total of 24 recruits survived to ten months, including 17 recruits on top surfaces, with an additional 261 *A. tenuis* recruits found on the natural reef substrata in the larval enhancement plots. Some of the larvae settled close together and after a few months some of these juveniles fused to form chimeras. After 25 and 27 months, a total of 14 fused colonies were recorded with each fused colony comprising of two to seven settlers.

From 10–34 months after settlement, the number of recruits on tiles declined slowly with 13 recruits alive on top surfaces after 25 months, and eight of those colonies surviving at 34 months (log-rank test, $\chi^2 = -255.24$, $P = 0.00$, top > sides = bottom;

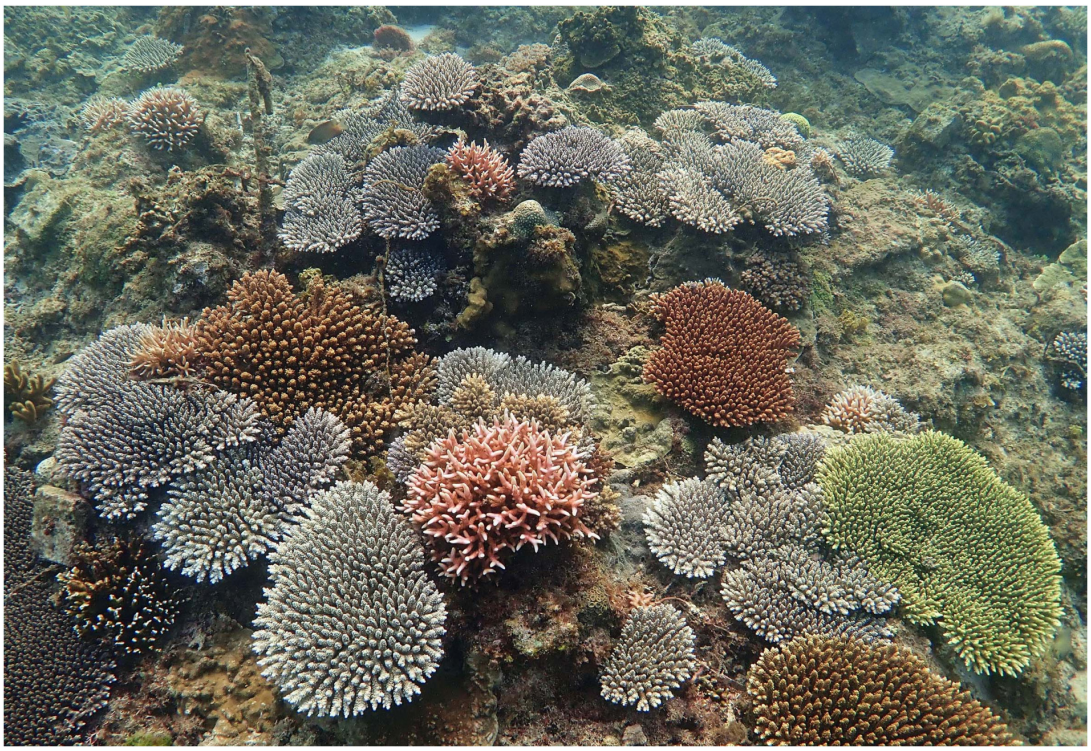

FIGURE 6 | High densities of *A. tenuis* colonies growing on a larval enhancement plot 34 months after larval settlement.

Figure 5B). The mean number of surviving colonies on tiles in each of the three larval plots after 34 months was 2.7 ± 0.67 per plot. Higher numbers of juvenile colonies resulting from larval settlement directly on reef substrata were found, with a total of 212 colonies alive in the three larval enhancement plots after 34 months. The mean number of surviving colonies on natural reef substrata in each larval enhancement plot at 34 months was 70.7 ± 16.83, equivalent to ∼5.7 colonies surviving per m^2 from larval settlement onto available reef areas in each larval enhancement plot (**Figure 6**).

Growth of Recruits and Onset of Sexual Reproduction

Growth of *A. tenuis* recruits, juveniles and colonies in the three larval enhancement plots was similar on recruitment tiles and natural reef substrata (**Figure 7A**), with no significant differences in average growth rates of colonies on tiles and reef surfaces (one-way ANOVA, $F = 3.29$, $P = 0.14$). Average growth rates of colonies on tiles from 10–34 months post-settlement were 90.8 ± 36.28 cm^3 mo^{-1}, and on reef substrata were 112.6 ± 17.55 cm^3 mo^{-1}. Repeated-measures MANOVA showed significant increases in coral volumes through time both on tiles and reef substrata in the larval enhancement plots ($F = 11.14$, $P = 0.000$), with no significant difference in volumes between substrata type ($F = 0.92$, $P = 0.392$). At 25 months after settlement (June 2018), average volumes of the fused colonies (2158.8 ± 333.02 cm^3 mo^{-1}) were 61% higher than those of individual colonies (824.1 ± 67.22 cm^3 mo^{-1}).

At 10 months post-settlement when recruits were visible on tiles and on the reef, the 24 recruits on the settlement tiles had a mean diameter of 1.4 ± 0.0 cm, and the 261 recruits on the reef substrata had a mean diameter of 2.4 ± 0.07 cm. At 34 months, the eight recruits on the settlement tiles had a mean diameter of 15.9 ± 3.12 cm, and the 212 recruits on the reef substrata had a mean diameter of 18.1 ± 0.82 cm (**Figure 7B**), with a size range from less than 5 cm up to > 25 cm mean diameter.

Reproductive condition of all colonies was assessed at 23 months' age, just prior to the potential first spawning period. Five colonies were gravid with pigmented eggs and well developed spermaries. These colonies ranged in size from 13.0 to 21.0 cm mean diameter (**Figure 8A**), and included one of the colonies growing on tiles, and four colonies growing on natural reef substrata in two of the larval plots. These colonies were observed spawning from 1830 to 1900 h on 1 and 2 May 2018 (1–2 nAFM, **Table 1**). Gametes collected from spawn cones placed over the gravid colonies were combined and transferred to the BML hatchery where high rates of fertilization were confirmed.

At 34 months, mean colony size for colonies on both tiles and reef substrata was 17.0 ± 1.86 cm and colony sizes ranged from less than 6 cm to more than 40 cm mean diameter (**Figure 8B**). A total of 77 colonies were sexually reproductive at this size and age, with gravid colonies ranging in size from 13.2 to 42.3 cm mean diameter (**Figure 8B**). These gravid colonies included four colonies growing on tiles and 73 colonies growing on natural reef substrata. Gravid colonies were observed spawning (**Figure 8C**)

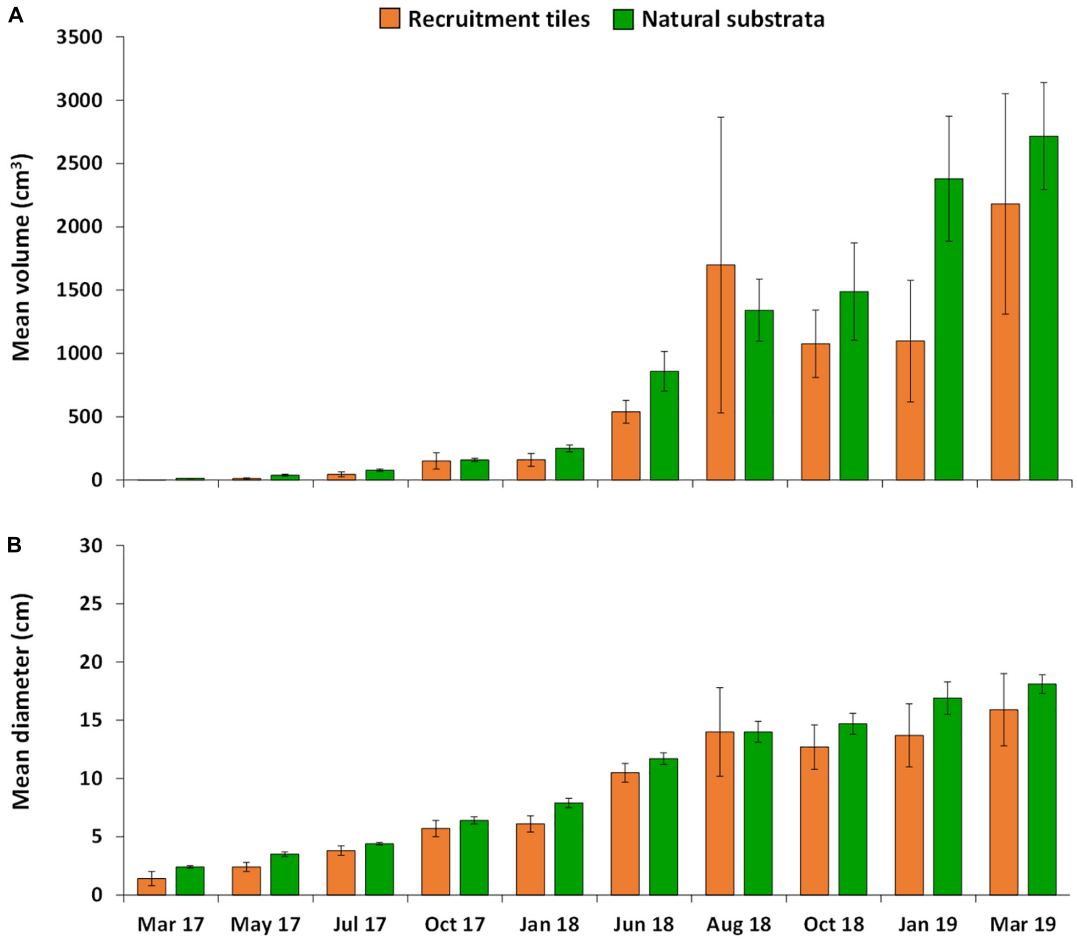

FIGURE 7 | Mean growth of *A. tenuis* colonies over 34 months in the three larval enhanced plots, **(A)** mean volume and **(B)** mean diameter of colonies on settlement tiles (orange) and natural substrata (green). Error bars are ± SE.

from 1830 to 1900 h on 23 and 24 May 2019 (4–5 nAFM, **Table 1**), and gametes were collected from the spawn cones and transferred into floating larval pool nets on the reef for larval culture.

Coral Production Costs

The production cost for each of the 285 sexually derived *A. tenuis* coral colonies alive at 10 months was United States $13.73. At 34 months age, the production costs for each of the 220 colonies in the restored breeding population was United States $17.79 (**Supplementary Table 1**).

Changes in Reef Community Status 2016–2019

Reef Benthic Community

In 2016, reef benthic cover and coral community status were very similar in the three larval enhancement plots and the three control plots (**Figure 9A**) with no significant differences in community structure (ANOSIM, R: 0.04, P = 0.30). All plots were degraded and characterised by low mean cover of living scleractinian corals (18.5% ± 2.04%), with similar mean cover of macroalgae and very low cover of soft corals, sponges, other invertebrates and dead coral covered with turf algae, together comprising 21.4% ± 5.72% mean benthic cover. Mean cover of dead coral substrata and coral rubble surfaces potentially available for coral larval settlement was 50.9% ± 6.86%, which represents about 12.5 m^2 of the reef area within each of the 25 m^2 plots (**Figure 9A**).

The 2016 coral community had low mean cover of *Acropora* spp. and higher cover of encrusting *Montipora* spp., with low cover of Pocilloporidae, Poritidae, Merulinidae and other taxa (**Figure 9B**). There were no significant differences in mean cover of *Acropora* (R: −0.14, P = 0.10), *Montipora* (R: −0.14, P = 0.60), *Porites* (R: −0.14, P = 0.10), Pocilloporidae (R: −0.04, P = 0.80) or Merulinidae (R: −0.14, P = 0.10) between larval enhanced and control plots prior to the larval restoration experiment.

In March 2019, coral cover had increased 35 months after larval restoration (**Figure 9C**), with the larval enhancement plots having significantly higher mean cover than controls (univariate PERMANOVA: F = 14.16, P = 0.0001), primarily due to the 9.5% ± 1.30% increase in *A. tenuis* mean cover from the larval restoration. Total mean cover of reef corals increased from 19.6% ± 3.12% in 2016 to 40.5% ± 6.13% in 2019 in the

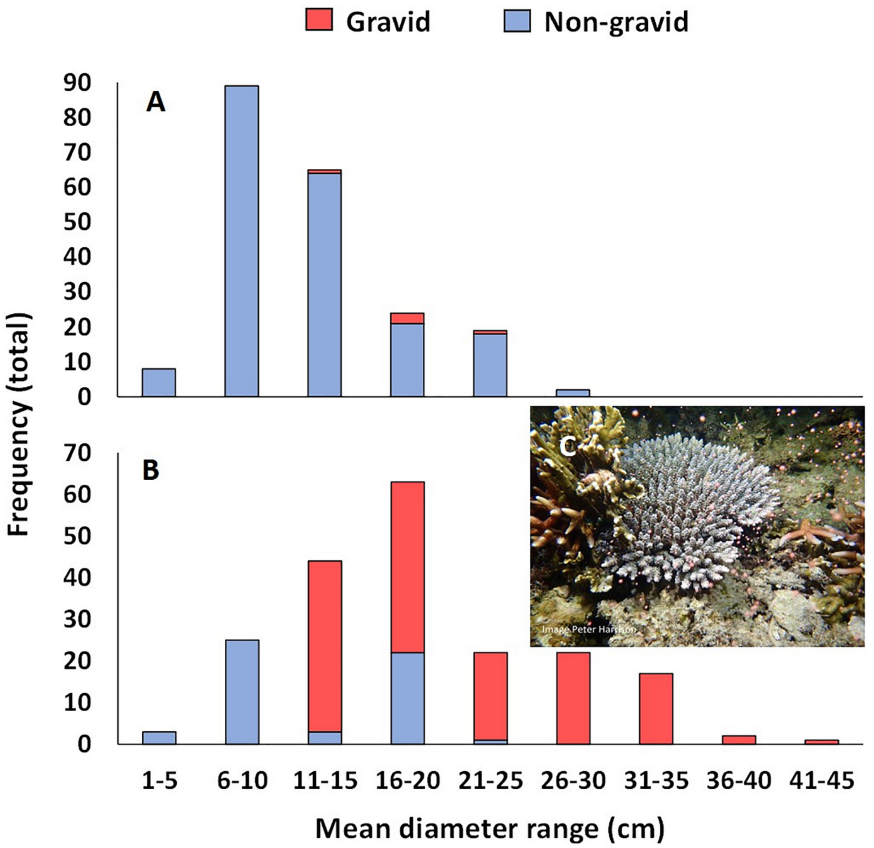

FIGURE 8 | Size frequency plots of mean colony size of gravid and non-reproductive *A. tenuis* colonies in the three larval enhanced plots **(A)** in June 2018 and **(B)** March 2019, and **(C)** a three year old colony spawning in a larval restoration plot on 25 April 2019.

larval enhancement plots, and from 17.3% ± 0.94% in 2016 to 26.2% ± 5.01% in 2019 in the control plots. Mean cover of non-scleractinian benthic categories were comparable to 2016 levels except for a reduction in macroalgae cover and hard substrata, corresponding with the increased cover of reef corals (**Figure 9C**).

Three years after larval restoration, *A. tenuis* cover had increased significantly in the larval enhancement plots compared with control plots (univariate PERMANOVA: F = 158.88, P = 0.0001) in which no new colonies of this species were recorded (**Figure 9D**). Mean cover of encrusting *Montipora* spp. had increased substantially in both larval enhancement (univariate PERMANOVA: F = 189.16, P = 0.0001) and control plots (univariate PERMANOVA: F = 3.73, P = 0.05) (**Figure 9D**). Mean cover of other reef corals was similar between surveys in 2016 and 2019 in both larval enhancement and control plots (**Figures 9B,D**).

Reef Fish Assemblage

Surveys of fish assemblages in 2016 showed similar low mean fish abundance and species richness in all experimental plots before the larval restoration experiment due to the degraded status of the reef. There were no significant differences between control and larval enhancement plots for fish abundance (**Figure 10A**; F = 2.7; P = 0.54) or fish species richness (**Figure 10B**; F = 1.86, P = 0.70) prior to the larval enhancement experiment. Pomacentridae and Labridae were the most abundant fish families, and had similar mean abundance in the larval enhanced and control plots in 2016, with low numbers of Chaetodontidae corallivores present (**Figure 11**).

Mean fish abundance and species richness varied slightly between larval enhanced and control plots and among years. Mean fish abundance increased in the larval enhancement plots in 2018 (**Figure 10A**) and was significantly higher compared to mean abundance in control plots in 2018 (Mann-Whitney U-Test: Z = −1.9640, P = 0.0463). There were no significant differences in fish abundance between larval enhancement and control plots in 2016, 2017, or 2019. Mean fish species richness was slightly higher in larval restoration plots than in control plots in 2018 and 2019 but these differences were non-significant (**Figure 10B**). The slight increase in fish abundance and change in reef fish assemblages between monitoring years is partly attributable to the increase in Pomacentridae (**Figure 11B**), specifically the turf farmer pomacentrids such as *Pomacentrus burroughi*, *P. chrysurus*, and *Plectroglyphidodon lacrymatus*.

Reef fish assemblages were similar in all plots, although assemblages in larval enhancement plots were mostly clustered separately from the control plots in each of the monitoring years (**Figure 12**). However, reef fish assemblages recorded in

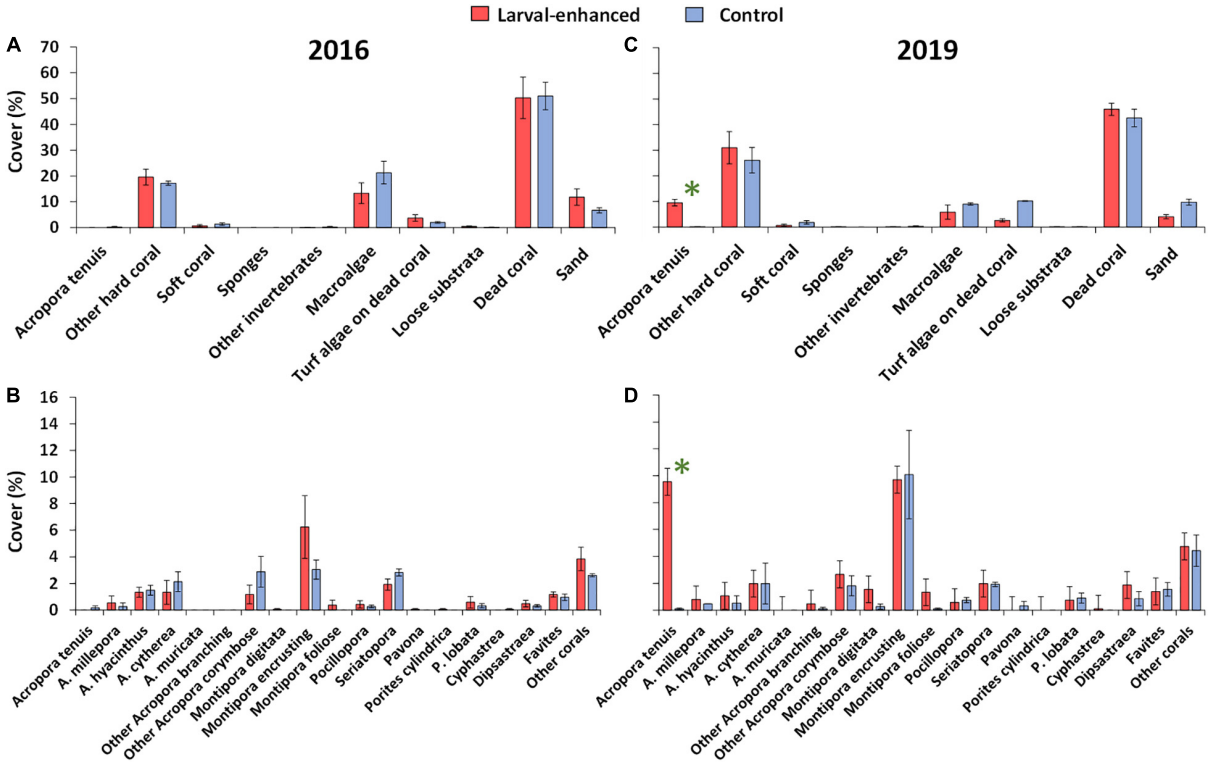

FIGURE 9 | Mean percentage cover of major benthic categories prior to larval enhancement in 2016 **(A)** and 35 months after larval enhancement in 2019 **(B)**, also mean percentage cover of coral categories prior to larval enhancement in 2016 **(C)** and 35 months after larval enhancement in 2019 **(D)**. Error bars are ± SE. *N* = 3 for both larval enhanced and control plots. Asterisk denotes significant difference in mean cover of *A. tenuis* between larval-enhanced and control plots.

2016 were significantly different (R = 0.466, P = 0.001) from the assemblages recorded in 2017, 2018, and 2019 (**Figure 12**). Pairwise comparisons showed significant differences between 2016 and each of the subsequent years (P = 0.01), but no significant differences between the years 2017, 2018, and 2019. In addition to the turf farmer fishes, pomacentrids that take refuge within *Acropora* coral branches such as *Dascyllus reticulatus*, *Chromis viridis*, and *Amblyglyphidodon curacao* contributed to the differences in reef fish assemblages in 2016 and later years.

A conceptual diagram summarising *A. tenuis* recruitment and growth, and changes in coral cover and reef fish assemblages during the three years following the 2016 larval restoration at Magsaysay reef is provided at **Figure 13**.

Spawn Capture and Larval Rearing on Magsaysay Reef

Large scale multi-species synchronous coral spawning events were observed *in situ* on night dives around a remnant "coral garden" reef patch with high coral cover and species richness on Magsaysay reef in 2016, 2017, and 2018, with coral spawn slicks forming at the sea surface on peak spawning nights (**Table 2**).

The spawning events noted in **Table 2** typically involved *Acropora* spp. colonies "setting" egg and sperm bundles under the inflated oral disc of polyps (*sensu* Harrison et al., 1984) from ∼1940 h, with buoyant bundles starting to be released synchronously from polyps by ∼2020 h, and spawning of some colonies occurring up to 2200 h and later. The largest coral spawn slicks developed on peak coral spawning nights (10–11 nAFM) that coincided with calm weather with low wind speeds and swell. As wind speed increased, spawned egg-sperm bundles, gametes and the slicks became dispersed across the sea surface.

These predictable large scale spawning events provided ready access to hundreds of millions of gametes from many colonies of diverse coral species, enabling development of new techniques and equipment for spawn collection and mass embryo and larval culture directly on reefs. In the 2016 pilot study, millions of gametes were collected in the 5 × 5 m net system that was attached to a floating bamboo frame deployed above healthy coral communities on 31 March (**Figures 3A,B**). Healthy developing embryos were recorded within the culture pool 12 and 20 h after spawning indicating that the system provided suitable environmental conditions for larval rearing on the reef. Additional coral spawn was added to the pool after spawning on 1 April before increasing wind dispersed the spawn at the sea surface preventing further collection. Strong winds and heavy wave action began to damage one corner of the larval culture pool's bamboo frame early the next morning, causing most of the developing larvae to wash out of the net system. Consequently, later designs of the low-cost bamboo frames were cross-braced and strengthened and four bamboo frames and a prototype semi-submersed 2 × 2 m PVC pipe frame were stress-tested in sea trials

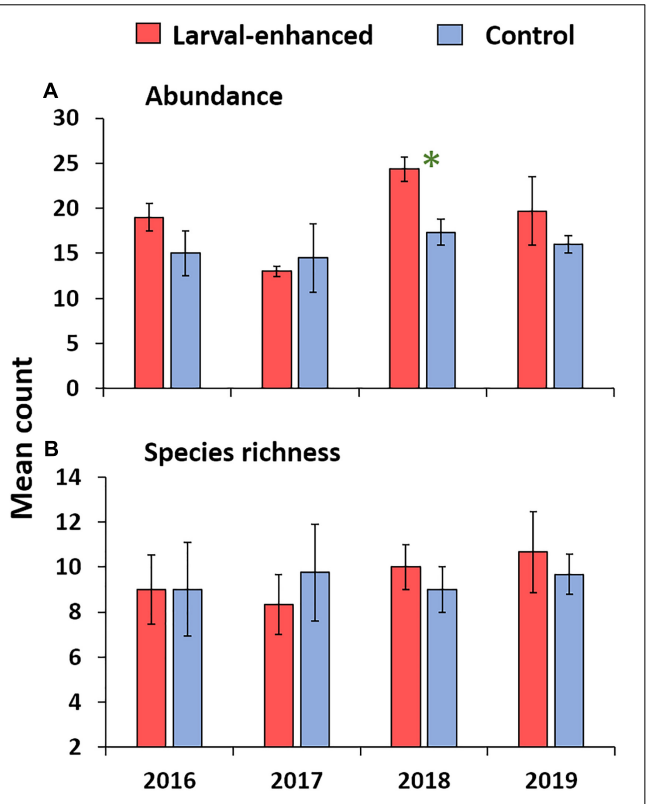

FIGURE 10 | Mean total fish abundance **(A)** and mean species richness **(B)** in larval enhanced (N = 3) and control (N = 3) plots before the larval restoration experiment in April 2016, and during annual surveys up to 2019. Error bars are ± SE. Asterisk denotes significant difference in mean fish abundance between larval-enhanced and control plots during 2018.

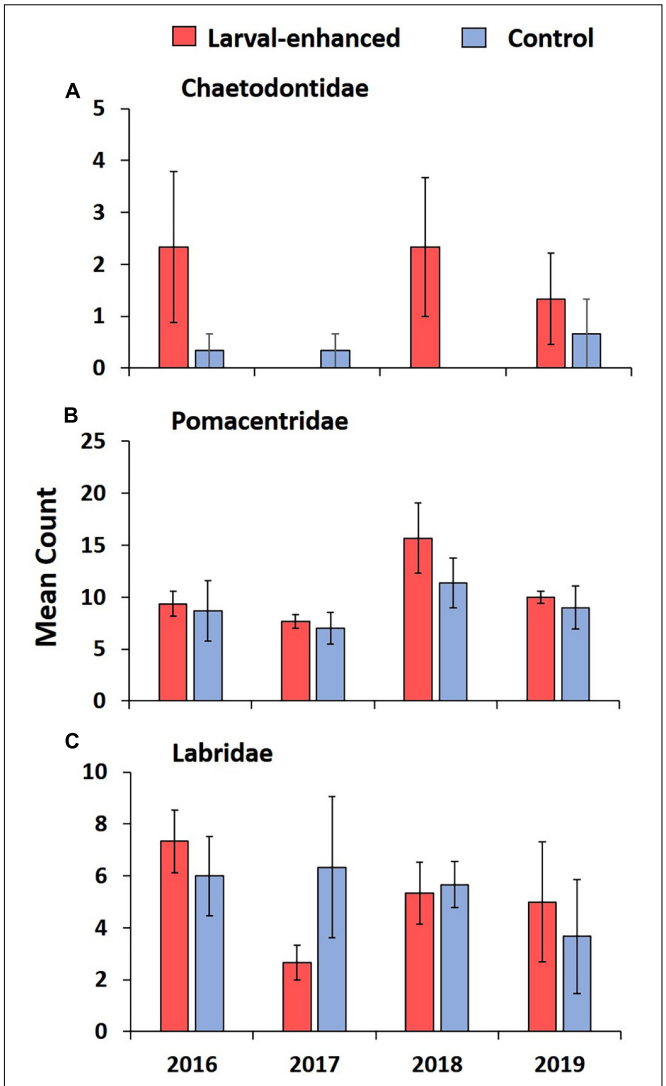

FIGURE 11 | Mean abundance of three commonly occurring fish families **(A)** Chaetodontidae, **(B)** Pomacentridae, and **(C)** Labridae in larval enhanced (N = 3) and control (N = 3) plots before the larval restoration experiment in April 2016 and during annual monitoring surveys until 2019. Error bars are ± SE.

while anchored near Magsaysay reef. These frames remained intact after three weeks including intermittent periods of strong winds and heavy wave action.

The at-sea larval collection and culture process were re-designed in 2017 (**Figure 3C**). Spawn slick samples were collected after the major spawning on 10 nAFM in March 2017, and an estimated 317,000 larvae were reared in the larval culture pool net enclosure supported within a 5 × 5 m steel frame with drum floats, which was temporarily moored adjacent to Magsaysay reef.

In 2018, the rearing pool net was further refined (**Figure 3D**), allowing for release of larvae directly from the net onto target reef sites by opening the zippered base. Coral spawn slicks were captured within the spawn catcher and larval pools after major spawning events on 12th and 13th March 2018, with > 90% fertilization rates recorded in samples.

DISCUSSION

Coral Larval Enhancement

Planning Restoration Interventions

Coral restoration should aim to re-establish breeding coral populations on damaged reefs using methods that are cost-effective and scalable, with restored populations capable of surviving and adapting to altered environmental conditions and stressors (Harrison, 2021). However, before restoration is attempted on any reef site it is important to use a decision framework to evaluate the need for such active interventions, and the likelihood of success using appropriate methods.

Initial baseline surveys are important to establish the status of reef communities, and determine the likelihood of natural recruitment enabling recovery within an appropriate timeframe without intervention. If the reef system is degraded and has very low natural recruitment and therefore unlikely to recover naturally, then active intervention is warranted if environmental conditions are potentially suitable for restoring coral populations in a cost-effective manner. Magsaysay reef, chosen for this study, is badly degraded with an algal phase-shifted reef community

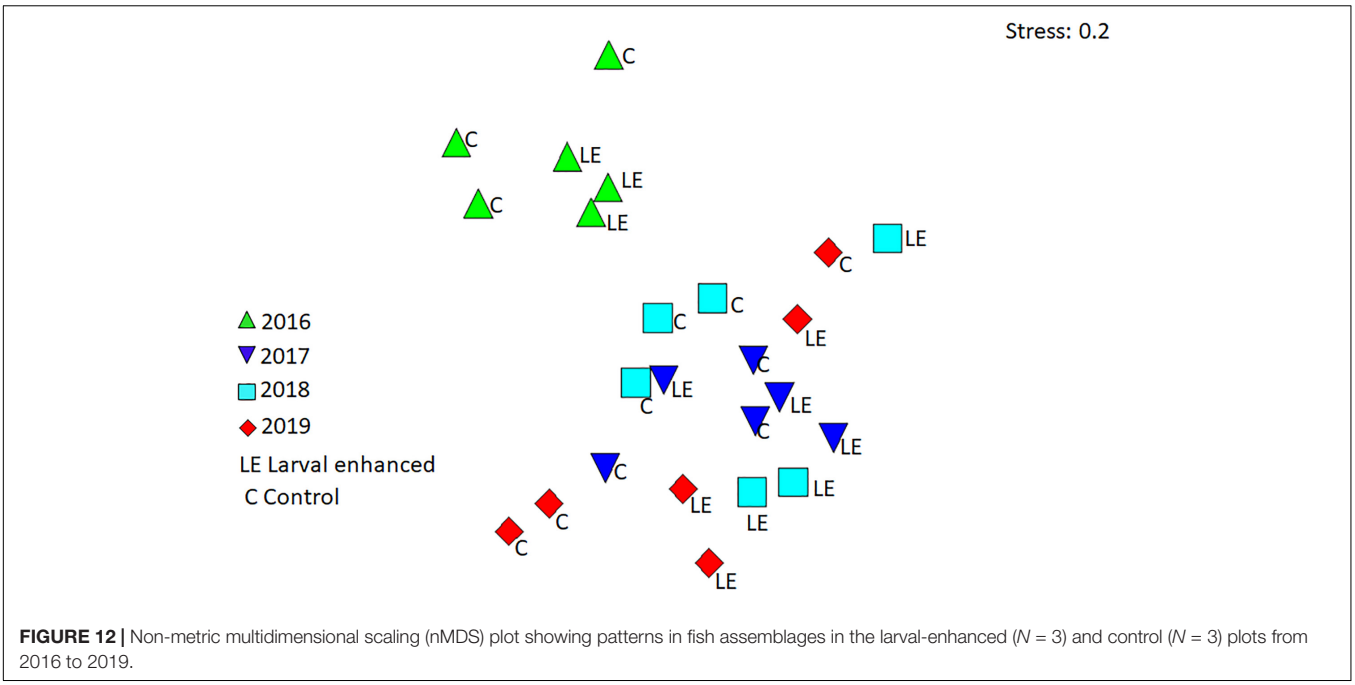

FIGURE 12 | Non-metric multidimensional scaling (nMDS) plot showing patterns in fish assemblages in the larval-enhanced (N = 3) and control (N = 3) plots from 2016 to 2019.

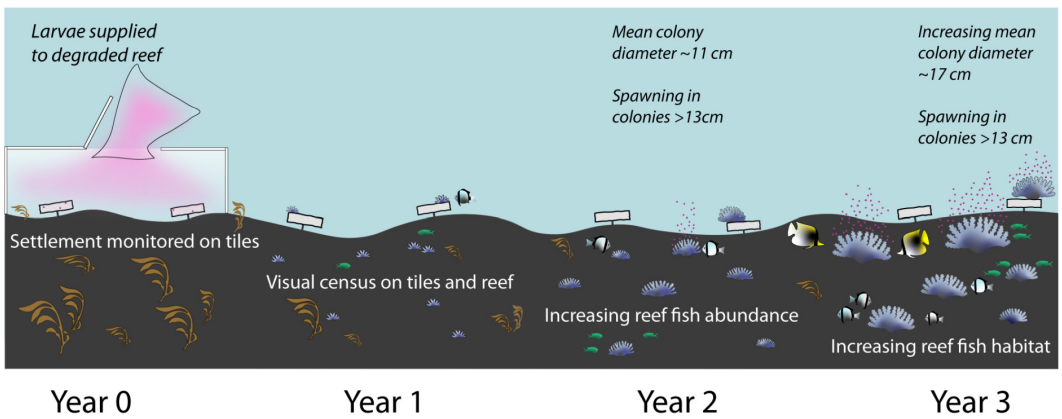

FIGURE 13 | Conceptual diagram showing changes in coral cover and reef community assemblage in the three years following the 2016 larval restoration at Magsaysay reef.

now characterised by low mean cover of live corals and few natural recruits, as it was in the 2013 larval restoration pilot study (dela Cruz and Harrison, 2017). That study also showed natural recruitment rates were low, and dominated by brooded pocilloporid spat with minimal *Acropora* recruits present on > 300 recruitment tiles deployed during a two-year period. Therefore, Magsaysay reef is unlikely to recover naturally without intervention.

The decision framework should also consider whether degraded reef systems are potentially recoverable, and the extent to which stressors and key threats that led to coral decline and reef degradation are still operating, or can be potentially managed or tolerated by new generations of restored corals. Previous key threats in the Bolinao-Anda Reef Complex (BARC) where this study was conducted included destructive fishing that is now controlled, a crown-of-thorns starfish corallivore outbreak in 2007 which is unlikely to re-occur given the low coral cover, and intermittent heat-stress and coral bleaching that had minimal impact on the 2013 restored *A. tenuis* population (dela Cruz and Harrison, 2017). Another key consideration for restoring degraded reefs is the extent to which the reef system is severely phase-shifted with low functional herbivory. Results from the 2013 pilot study showed that although the Magsaysay reef site is algal-dominated, enhancing larval supply significantly increased *A. tenuis* recruitment on larval restoration plots and restored a breeding population after three years (dela Cruz and Harrison, 2017). Therefore, Magsaysay reef is potentially recoverable, and restoring corals through increasing supply of sexually produced coral larvae can enhance genetic diversity and evolutionary potential, potentially improving

environment-genotype matching and resilience of surviving corals (van Oppen et al., 2017).

Larval Settlement and Recruit Survival

Our results confirm that mass larval enhancement can significantly increase the initial settlement of corals even on degraded reefs where natural larval supply and coral recruitment have been compromised by loss of adult breeding corals and high turf and macroalgal cover. In this study, we used ∼3.7 times higher supply densities of coral larvae than in the 2013 larval enhancement pilot study (dela Cruz and Harrison, 2017), which resulted in about eight times higher mean initial larval settlement per tile in 2016 compared with 2013. This is consistent with Cameron and Harrison (2020), who found a strong positive relationship between increasing larval density and total larval settlement. Higher rates of settlement in the present study therefore reflect the higher larval supply densities in plots and may also have been enhanced by the use of 7–8 day old larvae that were more fully developed and potentially primed for rapid settlement when released onto the reef areas, compared with the 4 day old larvae used in 2013. Most of the newly settled spat in the 2016 study had well-developed skeletons visible through the translucent polyp tissues (**Figure 4B**), indicating that the larvae probably settled rapidly after being released into the mesh enclosures on the reef. In addition, the mesh tent enclosures may have allowed larvae to actively swim and search for suitable settlement sites more effectively than under the flat mesh sheets used in the 2013 study. No *A. tenuis* recruits settled on tiles in control plots covered in mesh enclosures or on the 12 tiles in open control areas without mesh, which confirms low natural larval supply during the larval settlement experiment, similar to previous pilot studies (dela Cruz and Harrison, 2017, 2020b).

Mortality of settled spat and juvenile colonies on tiles was highest during the first 10 months after settlement and then stabilised (**Figure 5**), consistent with the Type III survivorship curve reported for other broadcast spawning corals (Babcock, 1985; Wilson and Harrison, 2005; Vermeij and Sandin, 2008; Doropoulos et al., 2016; dela Cruz and Harrison, 2017, 2020b) and marine invertebrates (Keough and Downes, 1982; Roughgarden et al., 1985; Hunt and Scheibling, 1997). The causes of mortality of the microscopic newly settled polyps on tiles are not known, but previous studies have noted overgrowth and competition from other benthic biota including allelopathic effects of algae, predation, damage from herbivore grazing, and reduced water quality and runoff from nearby coastal communities contributing to juvenile mortality (Sammarco and Carleton, 1981; Harrington et al., 2004; Penin et al., 2010; Guest et al., 2014; dela Cruz and Harrison, 2017; Cameron and Harrison, 2020). High mortality during early post-settlement life stages may also be a consequence of newly metamorphosed settled polyps having insufficient energy reserves for survival after expending energy and resources for metamorphosis and the onset of skeletogenesis (Harrison and Wallace, 1990), prior to uptake of mutualistic Symbiodiniaceae.

Recruits were visible on tiles and on reef surfaces by 10 months and repeated monitoring showed high rates of survival up to 35 months, consistent with high survivorship patterns of visible *Acropora* spp. Recruits after colonies reached size-escape thresholds that were recorded in previous studies (Babcock, 1991; Raymundo and Maypa, 2004; Ritson-Williams et al., 2009; Doropoulos et al., 2012; dela Cruz and Harrison, 2017, 2020b). The survivorship pattern of *A. tenuis* recruits from larvae that settled directly on reef substrata in 2016 (**Figure 5**) was intermediate between the pattern recorded for *A. tenuis* recruits in 2013 (dela Cruz and Harrison, 2017) and for *A. loripes* recruits (dela Cruz and Harrison, 2020b), and substantially higher than for *A. digitifera* recruits on ceramic plugs outplanted onto reef areas in Palau after 5 and 11 months (Humanes et al., 2021).

Growth and Sexual Reproduction

Mean growth rates of *A. tenuis* recruits and juveniles were similar on tiles and on natural reef substrata, and both were higher than for recruits from the 2013 pilot study (dela Cruz and Harrison, 2017). These growth rates were higher than those recorded for

TABLE 2 | Observations of multispecific coral spawning events at Magsaysay reef, 2016–2018.

Year	Multispecific spawning nights	Species observed spawning	Notes
2016	3–5 March (10–12 nAFM)	*A. cytherea* *A. digitifera* *A. florida* *A. gemmifera* *A. nana* *A. humilis* *A. hyacinthus* *A. latistella* *A. millepora* *A. sarmentosa* *A. samoensis*	Multiple colonies recorded spawning from 2020 h to after 2130 h. Peak spawning occurred on 5th March (12 nAFM), resulting in large coral spawn slicks on the sea surface.
	31 March–1 April (8–9 nAFM)	*A. florida* *A. humilis* *A. hyacinthus* *A. intermedia* *A. sarmentosa*	A second split-spawning (*sensu* Willis et al., 1985) was recorded after the second full moon in March.
2017	19–22 March (8–11 nAFM)	*A. cytherea* *A. digitifera* *A. florida* *A. hyacinthus* *A. intermedia* *A. latistella* *A. millepora* *A. muricata* *A. samoensis* *A. sarmentosa* *A. humilis* *A. valida*	Peak spawning occurred on 21 March (10th nAFM) resulting in a large coral spawn slick at the sea surface. Some colonies of *A. cytherea* and *A. hyacinthus* had mature gametes after these spawning periods, and these were subsequently observed spawning on 30–31 March (18–19 nAFM).
2018	12–15 March (10–13 nAFM)	*A. cytherea* *A. digitifera* *A. florida* *A. humilis* *A. hyacinthus* *A. millepora* *A. samoensis* *A. sarmentosa* *A. verweyi*	Large coral spawning events recorded on 12th and 13th March (10–11 nAFM), resulting in a large coral spawn slick at the sea surface. Smaller spawning events recorded on 14th March and 15th March.

A. tenuis settled in a hatchery and then outplanted onto reef areas after 18 months in Akajima, Japan (Iwao et al., 2010), and for *A. millepora* colonies grown in the BML hatchery or outplanted as sub-adults onto BARC reef areas in Northern Luzon, Philippines (Baria et al., 2012; Guest et al., 2014). Growth rates recorded during the present study were also higher than for *A. loripes* colonies growing from larvae settled directly in larval enhancement plots on Magsaysay reef (dela Cruz and Harrison, 2020b), and for *A. digitifera* colonies settled in an *ex situ* nursery and outplanted at five or 11 months (Humanes et al., 2021).

The rapid growth of most *A. tenuis* colonies resulting from direct larval settlement on reef areas indicates that environmental conditions are still suitable for survival and growth of this species on Magsaysay reef, despite its badly degraded status. Sea temperatures during this study ranged from 26.5 to 31°C (**Supplementary Figure 1**) and remained below the coral bleaching thresholds observed previously at Magsaysay reef (dela Cruz and Harrison, 2017). Seasonal changes were also evident in other environmental parameters including periods of reduced salinity and high turbidity from rainfall and runoff associated with monsoon conditions (**Supplementary Figure 2**). It is possible that elevated organic inputs and nutrients from seepage and runoff from adjacent coastal towns onto these nearshore reef systems are providing supplementary allochthonous food resources that are enhancing heterotrophic particulate feeding and dissolved organic matter uptake (Sorokin, 1993; Anthony, 2000), supplementing energy supplied from photosymbionts and predation on plankton. Larval settlement behaviour and suitable microhabitat selection directly on the reef may also have contributed to rapid growth of surviving colonies in comparison with other *Acropora* colonies reared from larvae that were settled in nurseries and subject to artificial environmental conditions and selection pressures during early life stages (Iwao et al., 2010; Baria et al., 2012; Guest et al., 2014).

The high densities of larvae supplied to the larval enhancement plots resulted in gregarious settlement of some larvae, and subsequent growth and fusion resulted in 14 chimeric colonies consisting of between two and seven individuals after two years. Chimerism has been reported among populations of *Acropora* species on reef areas (Puill-Stephan et al., 2009; Schweinsberg et al., 2015) and in experimental studies (dela Cruz and Harrison, 2017; Doropoulos et al., 2017; Cameron and Harrison, 2020; Sampayo et al., 2020), and may lead to enhanced early growth and survival to larger size refugia with potential for increased adaptive potential to changing environmental conditions.

Rapid colony growth also resulted in early onset of sexual reproduction in five colonies (13 to 21 cm mean diameter) that had mature gametes at two years after settlement, and therefore oogenesis was likely to have been initiated about 9 months prior to this at smaller colony sizes (Wallace, 1985; Harrison and Wallace, 1990; Randall et al., 2020). This is the fastest growth to sexual reproduction yet recorded in *Acropora* corals, and more rapid than predicted based on previous studies of this species (Wallace, 1985; Iwao et al., 2010; dela Cruz and Harrison, 2017) and other *Acropora* (Baria et al., 2012). Most colonies were sexually reproductive at three years of age with mean colony sizes above 13 cm diameter, thereby re-establishing a functional breeding population on the degraded reef system. The variability in colony sizes among breeding colonies is likely to reflect individual phenotype and holobiont responses to environmental conditions on Magsaysay reef, and highlights the complexity of predicting the age and size of sexual reproduction among reef corals (Wallace, 1985; Harrison and Wallace, 1990; Randall et al., 2020).

The timing of *A. tenuis* spawning was consistent among years and populations with all colonies recorded spawning during the crepuscular period around sunset, similar to spawning records from the Great Barrier Reef (Harrison et al., 1984; Willis et al., 1985; Babcock et al., 1986) and elsewhere (Baird et al., 2021). The lunar periodicity of spawning varied slightly among years and between the reef and BML culture facility (**Table 1**). Variability in the lunar night of spawning has been recorded among many *Acropora* populations in various reef regions, whereas some other taxa such as Merulinidae exhibit more consistent lunar periodicity of spawning, indicating that coral taxa may respond to proximate cues and ultimate selective pressures in different ways (Babcock et al., 1986; Harrison and Wallace, 1990; Hoadley et al., 2016; Randall et al., 2020).

The rapid re-establishment of breeding populations is important for initiating recovery of degraded reefs. Success at localised scales, such as achieved in this study, creates ongoing opportunities to expand larval restoration efforts to adjacent reef areas in future, using some of the millions of gametes now released annually by this population. These breeding populations also contribute to the depleted natural larval supply in the Lingayen Gulf, and some of these genetically diverse larvae are likely to disperse to other reefs and enhance recruitment and reef connectivity at larger scales over time (Harrison, 2006; Jones et al., 2009; Randall et al., 2020). As these breeding colonies grow and their spawning biomass increases, they become more fecund and hence their ecological value increases. In addition, the high densities of breeding colonies established during this reef trial are likely to enhance fertilisation rates on Magsaysay reef from high sperm and egg concentrations following synchronous spawning events (Oliver and Babcock, 1992; Levitan and Petersen, 1995; Yund, 2000). Remarkably, we know very little about the densities of breeding corals required to maximise fertilisation and cross-fertilisation rates on reefs, therefore future restoration trials should consider not only the overall abundance and spatial scales of restored colonies but also their densities for optimising breeding success (Teo and Todd, 2018).

Production Costs and Scaling

Key issues for coral restoration that need to be resolved are production costs and scalability. The average production costs for each *A. tenuis* coral colony at 10 months was United States $13.70, and United States $17.80 for each of the 220 colonies in the restored breeding population at 34 months age. These costs were lower than for the 2013 pilot study United States $21.00 per colony at 35 months (dela Cruz and Harrison, 2017) and for *A. loripes* United States $35.00 at 35 months (dela Cruz and Harrison, 2020b), largely as a result of significantly increased larval supply and higher numbers of recruits and adult colonies

surviving in the present study. This indicates that the cost-effectiveness of larval restoration should increase as mass larval production increases for larger-scale delivery onto damaged reefs, as long as long as settlement and recruitment density is optimised to avoid negative density-dependent mortality effects (Doropoulos et al., 2017; Cameron and Harrison, 2020). These production costs per colony for direct larval settlement onto degraded reef areas are substantially lower than for colonies reared in nurseries for extended periods prior to outplanting on reefs. For example, the production costs for 2.5 year old *A. millepora* colonies initially settled and held in the BML nursery for 7–19 months before outplanting onto reef areas in Northern Luzon, Philippines ranged from United States $284 to $61, respectively (Guest et al., 2014), and for 2.5 year old *A. digitifera* recruits settled and held in a nursery in Palau for 5 and 11 months before outplanting (United States $227 and $49, respectively; Humanes et al., 2021). These simple production cost metrics do not take into account the growing ecological and socio-economic values of these restored breeding colonies, which provide critical habitats for fish and other reef organisms, and annual increases in fecundity and production of millions of larvae.

Effects on Benthic Communities and Reef Fish Assemblages

Baseline reef community surveys in 2016 showed that the Magsaysay reef experimental plots were characterised by low mean live cover of reef corals and high cover of algae, and reduced abundance and diversity of reef fish, consistent with other degraded algal phase-shifted reefs (Bruno et al., 2009; Cheal et al., 2010; Ceccarelli et al., 2018). Three years after larval restoration, mean coral cover had doubled in the restoration plots to 40% primarily due to the restored *A. tenuis* population and growth of encrusting *Montipora* colonies present in the plots prior to larval enhancement. Coral cover also increased in the control plots due to growth of encrusting *Montipora*, but *Acropora* cover and growth of other colonies was negligible, and no additional *A. tenuis* colonies recruited onto the control plots over the three years of monitoring. These results indicate low natural larval supply and recruitment, therefore reef recovery will require active intervention through increased larval supply to catalyse the recovery of the foundation coral communities.

The significant increase in coral cover resulting from coral larval restoration corresponded with, and likely influenced, some changes in reef fish assemblages through time. There was a small increase in pomacentrids that shelter within coral branches (Coker et al., 2014) and an increase in chaetodontids that mainly feed on coral polyps (Cole and Pratchett, 2011) in the larval restoration plots; trends not evident in the control plots. This suggests that the larval restoration treatment enhanced the availability of suitable coral habitats for some fish on these reef areas. Similarly, increased abundance and diversity of reef fish and macroinvertebrates have been reported on other BARC reef areas following outplanting of coral fragments to increase coral biomass and reef structure (Cabaitan et al., 2008; dela Cruz et al., 2014).

Overall, fish assemblages in all plots were characterised by relatively low abundance and diversity, and dominated by small bodied individuals, reflecting the degraded status of the reef site (Jones et al., 2004; Nash and Graham, 2016). Although there were no clear differences in abundance of common reef fish functional guilds and families between control and larval restoration plots, these assemblages varied through time, particularly between 2016 and subsequent monitoring years. As the larval restoration and control plots are located within 10–20 m of each other, the increase in branching coral cover in the former may have influenced the mobile fish assemblages in the control plots. In addition, although the Magsaysay reef plots are included in the designated Magsaysay MPA, there is no enforcement of no-take zones on the reef and some fishers continue to fish within the MPA, so ongoing fishing pressures are likely to affect the fish assemblages at the restoration sites. Further community engagement and education about the need to protect the Magsaysay MPA reef sites, combined with increased local management and enforcement of fishing restrictions, is needed to enable fish habitats and fish assemblages to more fully recover and provide increased fish resources to other reef areas nearby (McCook et al., 2010; Russ et al., 2015).

Spawn Slick Capture and Mass Larval Rearing on Reefs for Larger Scale Restoration

To effectively scale up larval restoration and produce hundreds of millions or billions of coral larvae, we need to develop larger scale reef-based larval culture methods that are cost-effective and adaptable to different reef environments. Large-scale multispecies spawning events occur on many reefs around the world and often result in the formation of coral spawn slicks at the sea surface (Harrison et al., 1984; Babcock et al., 1986; Harrison and Wallace, 1990; Randall et al., 2020; Baird et al., 2021). These slicks provide ready access to billions or trillions of gametes, and enable collection of slick samples for larval rearing on reefs (Heyward et al., 2002; Omori, 2005; Doropoulos et al., 2019; Harrison, 2021).

The development of the integrated spawn catcher and larger larval culture pools in this study enables simple routine collection of coral spawn slicks containing hundreds of millions of egg-sperm bundles from diverse species, and mass culture of larvae from a diverse range of corals for mass larval supply over larger reef areas in future. These larval pools provide an effective method for mass larval production directly on reefs without the high costs of maintaining larvae and settlers in nurseries or on large vessels, and the floating frames can be produced at low cost ∼United States $200.00 each from bamboo, which is readily available throughout SE Asia and other major reef areas. The prototype bamboo frame was impacted by strong winds and rough seas, but subsequent cross-bracing strengthened the frame making it suitable for deployment in reef environments. The steel frames cost about United States $600.00 each but are more robust and have been used for six years, so these can provide a more cost effective approach if larval restoration is likely to be done over multiple years and in more exposed reef conditions.

Conclusion and Future Upscaling

The results of this study confirm that increasing larval supply and direct settlement on degraded reef areas can rapidly re-establish a breeding population of *Acropora tenuis* within two to three years and lead to significantly increased coral cover on restoration plots compared with control reef plots reliant on depleted natural larval supply. The higher densities of larval supply used in this study significantly increased larval settlement, recruitment and production of adult corals at higher densities on larval restoration plots, and at reduced cost compared with earlier studies. In addition, the increased cover of branching coral colonies corresponded with increased abundance of pomacentrids reliant on sheltering in branches, and increased chaetodontid corallivores on the larval restoration plots. Ongoing artisanal fishing pressures in the Magsaysay MPA will need to be managed in order to increase the abundance of larger fish and spawning stocks within the restoration areas, to enhance "spill-over" effects into nearby reef areas.

The new techniques for *in situ* spawn slick collection and larval culture on reefs developed in this study will enable more cost-effective mass larval production for increased scales of larval supply and restoration over larger reef areas in future. Use of natural spawn slicks will also enable multi-species cultures for restoring more diverse coral communities rather than single species populations. High post-settlement mortality bottlenecks that constrain recruitment in corals can be overcome by supplying higher densities of competent larvae. This allows natural selection pressures to operate on larger populations of settlers to select for genotypes that are better adapted to altered reef conditions. In addition, pre-settlement of competent larvae onto suitable natural dead coral or manufactured settlement surfaces with appropriate microtopography and microbial communities within the larval culture pools prior to deployment onto reef restoration areas should also significantly increase post-settlement survival, as would co-culturing larvae with more thermally tolerant Symbiodiniaceae to increase energy supply after settlement. Together, these approaches will increase coral recruitment success leading to faster and more efficient restoration of coral communities at larger scales.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PH obtained grant funding, designed the settlement nets and conceived the larval restoration, spawn catcher and larval pool methods, and wrote the draft manuscript. PH and DC designed the study and monitored larval settlement and interpreted data. DC collected and analysed benthic, recruit survival and growth data, and produced figures. PC co-ordinated monitoring fish assemblages and analysed fish data. KC contributed to larval rearing and collection of growth data and figures. All authors contributed to field work, manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.750210/full#supplementary-material

Supplementary Figure 1 | Sea Surface Temperature record obtained from NOAA.

Supplementary Figure 2 | Environmental data in the experimental plots. * = no data.

Supplementary Table 1 | Summary of costs for larval enhancement and production of *A. tenuis* coral colonies.

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Genetic Diversity and Structure of Tropical *Porites lutea* Populations Highlight Their High Adaptive Potential to Environmental Changes in the South China Sea

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Global climate change and anthropogenic disturbance have significantly degraded biodiversity in coral reef ecosystems. The genetic potential and adaptability of corals are key factors used to predict the fate of global coral reefs under climate change. In this study, we used eight microsatellite loci to study the patterns of reproduction, genetic diversity, and genetic structure of 302 *Porites lutea* samples across 13° latitudes in the South China Sea (8.8644°N–22.6117°N). The results indicated that *P. lutea* reproduces largely via sexual reproduction on scales of 5 m and greater and has abundant gene diversity. Additionally, the tropical populations harbored high genetic diversity (based on alleles, effective number of alleles, gene diversity, and heterozygosity). In contrast, genetic diversity was lower in subtropical coral populations. Genetic variation values and pairwise F_{ST} revealed that tropical and subtropical populations had significantly different genetic structures. Finally, the Mantel tests showed that the genetic differentiation and genetic variation of *P. lutea* were strongly correlated with sea surface temperature and slightly correlated with geographical distance. These results indicated that tropical *P. lutea* populations have high genetic potential and adaptability because of their sexual reproduction and genetic diversity, giving them a greater capacity to cope with climate change. Subtropical coral populations showed lower genetic diversity and, thus, relatively poor genetic resilience in response to low average sea surface temperature and human activities. Our study provides a theoretical basis for the protection and restoration of coral reefs.

Keywords: *Porites lutea*, genetic potential, genetic diversity, genetic structure, sea surface temperature, climate change

INTRODUCTION

Global warming has greatly decreased biodiversity and poses a critical threat to the health of coral reef ecosystems (Hughes et al., 2017; Thomas et al., 2017). The rising sea surface temperature (SST) causes a breakdown in the symbiotic associations between the coral host and its endosymbiotic dinoflagellate, resulting in coral bleaching (Chen et al., 2018). For instance, the record temperatures

reported during 2015–2016 triggered a pan-tropical episode of coral bleaching (Hughes et al., 2017). An important factor in coral recovery from environmental stress is its genetic potential (Williams et al., 2014), or the ability of a population to evolve under changing selection pressures (Meyers et al., 2005). Genetic diversity was regarded as the fundamental evolutionary source in speciation and adaptation, reflecting the level of population genetic potential and adaptability. As a population's genetic diversity increases, so does its genetic potential (Barrett and Schluter, 2008; Hoegh-Guldberg and Bruno, 2010; Thomas et al., 2017). With increases in environmental stressors such as eutrophication, habitat fragmentation, and climate change, the genetic diversity of keystone species may become increasingly important (Hughes and Stachowicz, 2004). The sea surface temperature, ocean currents, breeding patterns, and geographical distances were the most reported factors affecting genetic characteristics of corals in some coral reefs (Knittweis et al., 2009; Tye et al., 2013; Mclachlan et al., 2020). In Western Australia, the low latitude coral populations had higher genetic potential than the high latitude coral populations. The corals in high latitudes were more vulnerable than corals in tropical areas to cope with global climate change (Ayre and Hughes, 2004; Miller and Ayre, 2010; Thomas et al., 2017). Genetic diversity is considered to enhance the sustainability of populations over evolutionary time scales by furnishing sufficient alleles for future environment changes (Ayre and Hughes, 2004; Williams et al., 2014).

The coral reefs of the South China Sea (SCS) are on the northern edge of the Coral Triangle region and comprise high diversity and abundant biological resources (Yu, 2012). The SCS contains both tropical and subtropical coral reefs (Yu, 2012), with distinct SSTs linked to latitudinal gradients (Chen et al., 2019; Qin et al., 2019). However, the genetic characteristics of corals have barely been investigated in the SCS. Recently, Wu et al. (2021) reported low genetic diversity and moderate genetic differentiation of the dominant coral *Turbinaria peltata* at relatively high latitudes in the SCS. The genetic structure was significantly affected by the average SST, geographical isolation, and anthropogenic activities. Huang et al. (2018) reported high genetic diversity and connectivity in *P. lutea* across the SCS based on nuclear markers (internal transcribed spacer and β-*tubulin*). However, no evident genetic structure or effecting factors were found. The genetic characteristics of *Mycedium elephantotus, Platygyra sinensis*, and *Platygyra verweyi* in the north SCS also have no obvious genetic structure (Yu et al., 1999; Ng and Morton, 2003; Keshavmurthy et al., 2012). The limited genetic information and limited number of species investigated in this area has hampered the formulation of coral reef protection measures.

Porites lutea is widely distributed in the subtropical and tropical regions of the SCS. This coral species is considered to be adaptable to climate change and other forms of anthropogenic disturbance (Xu et al., 2017; Qin et al., 2019). The species contains a high density of heat-tolerant symbiotic zooxanthellae C15 and a rich diversity of symbiotic microorganisms (Liang et al., 2017; Chen et al., 2020). In this study, we investigated the genetics of *P. lutea* from coral reef sites in the SCS spanning 13° latitudes based on eight microsatellite loci. We focused on (1) the relationship between genetic potential and reproduction patterns and genetic diversity, (2) how genetic structure varies across latitudes, (3) the relationship between *P. lutea* genetic structure and SST in the SCS, and (4) the genetic potential and adaptability of *P. lutea* populations in tropical areas.

MATERIALS AND METHODS

Sample Collection

Fragments (∼2–3 cm^2, $n = 302$) of *P. lutea* were collected from 14 different coral reef sites in the SCS, covering a wide range of latitudes (8.8644°N to 22.6117°N): Xiaolajia and Yangmeikeng in Daya Bay; one site in Weizhou Island; one site-Luhuitou in Sanya Bay; Beijiao, Qilianyu, Yongxing, Dongdao, Yuzhuo, and Langhua in the Xisha Islands; and Huangyan in Zhongsha Islands; Sanjiaojiao, Xinyijiao, and Dongjiao in the Nansha Islands (**Table 1** and **Figure 1**). We identified *P. lutea* based on morphological characteristics. In addition, the collected samples of *P. lutea* were confirmed by ITS in our previous study (Huang et al., 2018). Furthermore, many studies showed that *P. lutea* is widely distributed in the SCS (Yu, 2012; Huang et al., 2021). Fragments were obtained at depths of 4–10 m. Sampled colonies were separated by at least 5 m to minimize the probability of collecting samples from the same ramet more than once (Magalon et al., 2005). Small fragments were broken from colonies using a hammer and chisel, and then stored in 95% ethanol or at −80°C until DNA extraction. Genomic DNA was extracted using a marine animal tissue genomic DNA extraction kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol.

PCR Amplification and Sequencing

Microsatellite markers can be used to study the genetics of different species of the same genus (Magalon et al., 2004; Severance et al., 2010). Eight microsatellite loci from *P. lobata* were validated for the analysis of *P. lutea* in this study. Primers PL0340, PL1556, PL2258, and PL0780 were from Polato et al. (2010), and primers PL1370, PL0905, PL1483, and PL1868 were from Baums et al. (2012). The forward primer of PL0340, PL2258, PL0780, PL0905, PL1968 was fluorescently labeled with 6FAM. The forward primer of PL1556, PL1370, PL1483 was fluorescently labeled with HEX. The PCR was performed with 50 ng template DNA, 0.4 μL forward primer, 0.4 μL reverse primer, 10 μL 2× PCR enzyme mix (Tiangen Biotech) and Tiangen-free water to the total volume of 20 μL. Thermocycling (ABI GeneAmp® 9700) was performed as follows: 94°C for 3 min, 40 cycles at 94°C for 30 s, annealing temperature for 30 s, 72°C for 45 s, and 72°C for 5 min. Fragments were analyzed using capillary electrophoresis, on an ABI 3730 sequencer with an internal size standard Genescan LIZ 500 (Applied Biosystems). Electropherograms were visualized, and allele sizes were calculated in GENEMAPPER 4.0. Each marker

TABLE 1 | *Porites lutea* samples were collected from 14 coral reef sites in the South China Sea.

Region	Site	Reef type	*Sampling dates*	*N*	*Ng*	*Ng/N*	Latitude	Longitude
Daya Bay	Xiaolajia	Subtropical non-reefal	2018.05	18	18	1.00	22.6117°	114.6311°
	Yangmeikeng	Subtropical non-reefal	2018.05	21	21	1.00	22.5492°	114.5694°
Weizhou Island	Weizhou	Subtropical reefal	2015.08	20	20	1.00	21.0204°	109.0805°
Sanya Bay	Luhuitou	Tropical reefal	2015.08	20	20	1.00	18.2176°	109.4855°
Xisha Islands	Beijiao	Tropical reefal	2015.08	19	19	1.00	17.1033°	111.4830°
	Qilianyu	Tropical reefal	2015.08	23	23	1.00	16.9703°	112.3142°
	Yongxing	Tropical reefal	2015.08	24	24	1.00	16.8476°	112.3588°
	Dongdao	Tropical reefal	2015.08	23	23	1.00	16.6748°	112.7375°
	Yuzhuo	Tropical reefal	2015.08	23	23	1.00	16.3638°	112.0168°
	Langhua	Tropical reefal	2015.08	20	20	1.00	16.0845°	112.5921°
Zhongsha Islands	Huangyan	Tropical reefal	2015.08	23	23	1.00	15.2190°	117.7477°
Nansha Islands	Sanjiaojiao	Tropical reefal	2016.08	24	24	1.00	10.1899°	115.2967°
	Xinyijiao	Tropical reefal	2016.08	22	22	1.00	9.3281°	115.9332°
	Dongjiao	Tropical reefal	2018.06	22	21	0.95	8.8644°	112.8300°
Total				302	301			
				21.57	21.50	0.99		
SD				1.84	1.84	0.13		

Sample size (N), number of unique multilocus genotypes (Ng).

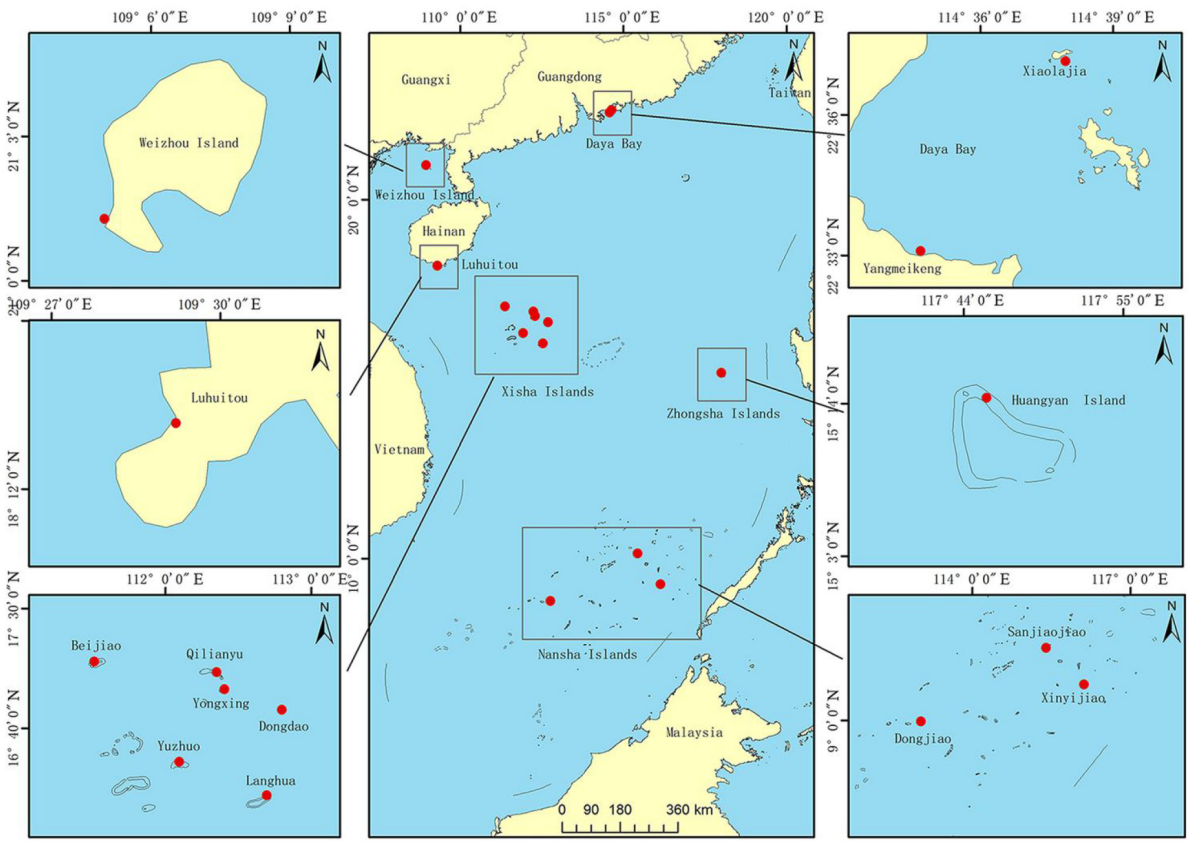

FIGURE 1 | Samples collected from sites in the South China Sea (SCS). Daya Bay is at the northern edge of *Porites lutea* distribution in the SCS, a region that also includes Xiaolajia and Yangmeikeng. Most coral colonies at Daya Bay have not developed into extensive coral reefs (Chen et al., 2007). Weizhou Island is in the Beibu Gulf at (northwestern SCS); colonies there have developed into extensive coral reefs. Sanya Bay is in the tropical region of the sea. Xisha Islands, Zhongsha Islands, and Nansha Islands are also part of the tropical region. Xisha Islands include Beijiao, Qilianyu, Yongxing, Dongdao, Yuzhuo, and Langhua. Nansha includes Sanjiaojiao, Xinyijiao, and Dongjiao.

was verified to ensure effective amplification of the DNA samples at each site.

Data Analysis

Unique multilocus genotypes (MLGs) were identified in GenAlex 6.502 (Peakall and Smouse, 2005), by requiring complete matches in all loci. The number of MLGs per population provides an estimate of *P. lutea* propagation patterns. GenAlex 6.502 was also used to analyze genetic diversity indices, including the average number of alleles per locus in the populations (*Na*) and average number of effective alleles per locus in the populations (*Ne*). The genetic variation was tested by Mantel test in GenAlex 6.502 (Peakall and Smouse, 2005). Observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were analyzed in MSA v4.0 (Dieringer and Schlötterer, 2003). FSTAT (Goudet, 1995) was used to calculate gene diversity (*Gd*) and pairwise F_{ST} ($F_{ST} < 0.05$, low genetic differentiation; $0.05 < F_{ST} < 0.25$, moderate genetic differentiation; $F_{ST} > 0.25$, high genetic differentiation). Analysis of molecular variance and principal coordinates analysis (PCoA) were conducted for the geographic populations using GenAlex 6.502 (Peakall and Smouse, 2005; Chen et al., 2020). Based on a matrix of covariance computed from allele frequencies, PCoA can visualize genetic relationships across different latitudes (Peakall and Smouse, 2005). Correlations between genetic structure and geographical distance as well as environmental factors (average SST and SST variance) were determined using Mantel tests, which were performed in IBD (Bohonak, 2002) with 1,000 randomizations. The geographic distances between *P. lutea* populations were determined using Google Earth version 4.3 and followed the shortest route from the waterway.

The genetic structure was analyzed in STRUCTURE 2.3.4, a program that can estimate the most likely number of genetic clusters (*K*) (Pritchard et al., 2000). The method on Delta K implement of program STRUCTURE is the widely and popular way to infer population genetic structure (Pritchard et al., 2000; Polato et al., 2010; Baums et al., 2012; Tay et al., 2015; Chen et al., 2020). STRUCTURE implements Bayesian cluster algorithms to assign genotypes to clusters that maximize deviation from Hardy-Weinberg equilibrium expectations and minimize linkage disequilibrium, providing an accurate representation of contemporary divergence (Hubisz et al., 2009). CONVERT (Glaubitz, 2004) was used to format the input data before using STRUCTURE. Correlated allele frequencies and admixed populations were also assumed. Values of $K = 1$–14 were analyzed by running replicate simulations ($\geq$3) with 10^6 Markov Chain Monte Carlo repetitions each, with a burn-in of 10^5 iterations (Evanno et al., 2005; Polato et al., 2010). Each K value was run 10 times in STRUCTURE. The *K* value based on the STRUCTURE output was determined using STRUCTURE HARVESTER (Earl and Vonholdt, 2012) to plot the log probability [$L(K)$] of data over multiple runs and to compare the outcome with delta *K* (Evanno et al., 2005). The results of the STRUCTURE run with the optimal *K* were merged with CLUMPP (Jakobsson and Rosenberg, 2007) and then visualized using DISTRUCT version 1.1 (Rosenberg, 2004). For all analyses, significance was set at $P < 0.05$.

RESULTS

Multilocus Genotyping

We verified that the selected microsatellite markers were suitable for our study. The locus failure rate was 0.33∼5.96%, with the lowest in PL0340 and the highest in PL1556, respectively (**Supplementary Table 1**). The overall missing loci in samples were 2.94%.

Porites lutea in the SCS showed high genotypic diversity. Genetic analysis of 14 *P. lutea* communities revealed 301 unique MLGs (**Table 1**). The Dongjiao coral reef site (Nansha Islands) possessed only one set of two identical MLGs (**Table 1**). This repeated *MLG* is attributable to a single common genotype occurring across distinct patches within each population. Nevertheless, the high proportion of unique MLGs (mean $Ng/N = 0.99 \pm 0.13$, **Table 1**) confirmed the substantial diversity of *P. lutea* genotypes, possibly because of sexual reproduction, and indicated considerable clonal richness.

Genetic Diversity of *Porites lutea*

In our study populations, *Na* ranged from 6.000 to 9.750 and *Ne* ranged from 3.899 to 6.415. Additionally, *Ho* ranged from 0.515 to 0.749, *He* ranged from 0.685 to 0.844, and *Gd* ranged from 0.632 to 0.839 (**Table 2**), According to the results (**Supplementary Table 2**), Fis ranged from −0.313 to 0.698. A small part of loci revealed significant deviation from HWE and was mainly found in Dongjiao population in Nansha Islands. These results for the alleles, effective number of alleles, gene diversity, and heterozygosity demonstrated that tropical *P. lutea* communities had high genetic diversity. Notably, subtropical *P. lutea* communities (particularly in Daya Bay) showed lower genetic diversity than in the tropical coral populations. In fact, tropical *P. lutea* were abundant, healthy, and formed reefs, whereas *P. lutea* in Daya Bay were scattered and did not form reefs.

Genetic Structure of *Porites lutea*

Coral populations from Daya Bay exhibited significant genetic variation compared to other tropical coral populations ($\Phi_{PT} = 0.129$–0.290, $P < 0.01$) (**Table 3**). In particular, the Xiaolajia population exhibited strong genetic variation between the Yuzhuo, Langhua and Dongjiao populations. The Weizhou Island population showed moderate genetic variation compared with tropical populations ($\Phi_{PT} = 0.085$–0.148, $P < 0.01$) (**Table 3**). Notably, the Luhuitou population exhibited slight variation from other tropical populations (in addition to Beijiao, Dongdao, and Xinyijiao). Therefore, the genetic structure of *P. lutea* is likely related to the latitudinal gradient.

Pairwise F_{ST} values derived from microsatellite markers showed that the Xiaolajia and Yangmeikeng subtropical populations of Daya Bay were significantly genetically

TABLE 2 | Genetic diversity of *Porites lutea* samples from the South China Sea estimated using eight microsatellite loci.

Site	*Na*	*Ne*	*Gd*	*Ho*	*He*
Xiaolajia	6.000	3.899	0.632	0.679	0.685
Yangmeikeng	6.125	4.304	0.735	0.515	0.758
Weizhou Island	6.875	4.670	0.792	0.728	0.795
Luhuitou	9.000	5.998	0.839	0.615	0.844
Beijiao	7.625	5.303	0.823	0.672	0.834
Qilianyu	9.750	6.415	0.819	0.624	0.835
Yongxing	8.625	6.095	0.807	0.648	0.825
Dongdao	8.750	6.153	0.815	0.669	0.828
Yuzhuo	8.500	5.497	0.787	0.747	0.803
Langhua	7.250	4.928	0.784	0.732	0.798
Huangyan	8.125	5.996	0.783	0.569	0.802
Sanjiaojiao	9.250	5.987	0.813	0.602	0.824
Xinyijiao	8.875	6.254	0.827	0.602	0.837
Dongjiao	7.875	5.509	0.771	0.749	0.797

Data were analyzed based on the individual site and locus. Na, average number of alleles observed; Ne, average number of effective alleles per locus in populations; Gd, gene diversity; Ho, average observed heterozygosity; He, average expected heterozygosity.

differentiated from tropical coral populations (F_{ST} = 0.1170–0.2361, $P < 0.05$). These Daya Bay populations differed significantly from tropical populations (**Supplementary Table 3**). The results of PCoA indicated that the *P. lutea* populations formed two genetic clusters: subtropical populations and tropical populations (**Figure 2**). Notably, population of Weizhou Island was slightly isolated from the Daya Bay populations (**Figure 2**).

Cluster Analysis of *Porites lutea*

According to plots of Delta K (**Supplementary Figure 1**) and LnP (D) (**Supplementary Figure 2**) from STRUCTURE analysis, the 14 *P. lutea* populations could be grouped into two genetic clusters with the optimal $K = 2$ (**Figure 3**). The subtropical populations of Daya Bay and Weizhou Island, and tropical populations formed two distinct and closely knit genetic units (**Figure 3**). The green cluster was found at high frequencies in samples collected from Daya Bay and Weizhou but with lower frequencies in samples from other coral reef sites. But green and little red clusters were found on Weizhou Island. The green clusters with a lower frequency were found on tropical populations. The red clusters were mainly found on tropical populations.

Correlations Between Environment Factors and Genetic Structure of *Porites lutea*

We acquired SST data covering January 1982 to December 2018 from the KNMI Climate Explorer[1] (**Figures 4A,B**). Initial analysis indicated that the subtropical and tropical regions widely varied in seasonal SST. The monthly average SST was low in the subtropical coral reefs of Daya Bay and Weizhou Island. In the tropical regions, including the Xisha Islands, Zhongsha Islands, and Nansha Islands, the SST was relatively stable and high (**Figures 4A,B**).

The results of the Mantel tests showed that genetic variation (Φ_{PT}) was positively correlated with the average SST ($R^2 = 0.3995$, $P = 0.001$), SST variation ($R^2 = 0.3860$, $P = 0.001$), and geographic distance ($R^2 = 0.2358$, $P = 0.003$, **Figure 5**). Geographic distance had a relatively small impact on genetic variation. In addition, F_{ST} was positively correlated with the average SST ($R^2 = 0.3764$, $P = 0.001$), SST variation ($R^2 = 0.3525$, $P = 0.001$), and geographic distance ($R^2 = 0.2105$, $P = 0.003$, **Supplementary Figure 3**). This result was consistent with that of Φ_{PT}. Therefore, SST and geographic distance were the

[1]http://climexp.knmi.nl/start.cgi

TABLE 3 | Pairwise Φ_{PT} values of *Porites lutea* in the South China Sea.

Φ_{PT}	XLJ	YMK	WZ	LHT	BJ	QLY	YX	DD	YZ	LH	HY	SJJ	XYJ	DJ
XLJ														
YMK	0.047													
WZ	**0.192**	**0.121**												
LHT	**0.211**	**0.129**	**0.085**											
BJ	**0.232**	**0.157**	**0.119**	0.039										
QLY	**0.217**	**0.139**	**0.107**	**0.051**	0.022									
YX	**0.212**	**0.145**	**0.090**	**0.057**	0.015	0.001								
DD	**0.238**	**0.161**	**0.090**	0.040	0.022	0.006	0.014							
YZ	**0.289**	**0.223**	**0.148**	**0.098**	**0.054**	0.020	0.042	0.028						
LH	**0.271**	**0.196**	**0.103**	**0.066**	**0.064**	**0.056**	**0.051**	**0.057**	**0.096**					
HY	**0.232**	**0.147**	**0.130**	**0.054**	0.031	0.009	0.025	0.033	0.033	**0.076**				
SJJ	**0.226**	**0.147**	**0.115**	**0.058**	0.025	0.007	0.006	0.027	0.043	**0.065**	0.006			
XYJ	**0.218**	**0.149**	**0.117**	0.047	**0.054**	0.012	0.013	0.035	0.047	**0.062**	0.030	0.005		
DJ	**0.290**	**0.210**	**0.145**	**0.088**	**0.066**	0.045	0.047	**0.055**	**0.051**	**0.070**	**0.061**	**0.054**	**0.067**	

Significant Φ_{PT} values (sequential-Bonferroni-corrected $P < 0.05$) marked in bold. Xiaolajia (XLJ), Yangmeikeng (YMK), Weizhou Island (WZ),Luhuitou (LHT), Beijiao (BJ), Qilianyu (QLY), Yongxing (YX), Dongdao (DD), Yuzhuo (YZ), Langhua (LH), Huangyan (HY), Sanjiaojiao (SJJ), Xinyijiao (XYJ), Dongjiao (DJ).
The bold values mean significant ϕ_{PT}.

FIGURE 2 | Principal coordinate analysis (PCoA) of samples from the South China Sea.

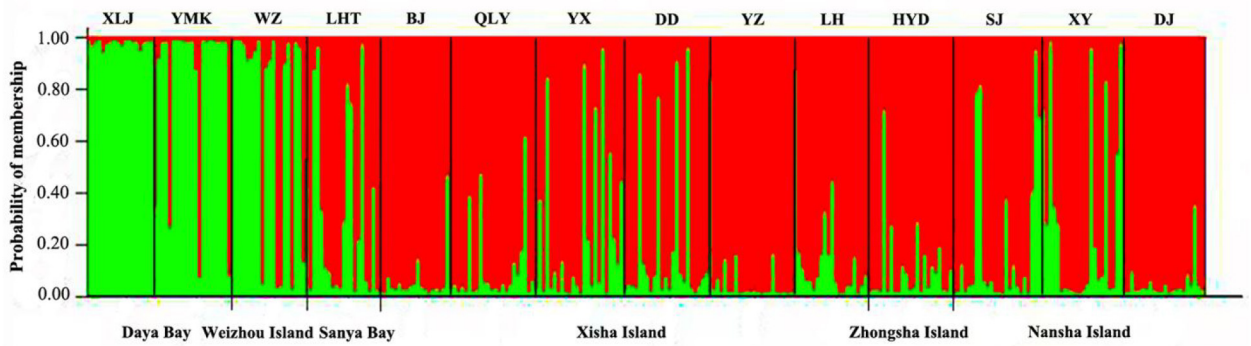

FIGURE 3 | Cluster analysis of *Porites lutea* in the South China Sea performed using STRUCTURE (optimal $K = 2$). The *y*-axis indicates the membership probability of each site ($n = 14$) in distinct population clusters (different colors). Sampling sites are identified along the *x*-axis.

dominant factors determining the genetic structure of *P. lutea* in the SCS. The SST had a stronger influence compared with geographic distance.

DISCUSSION

Tropical *Porites lutea* Populations May Have High Adaptive Potential

Using microsatellite markers, we identified 301 unique MLGs in 14 *P. lutea* populations. This result suggests that *P. lutea* reproduces largely via sexual propagation on scales of 5 m and greater and has abundant gene diversity. The genetic diversity of tropical *P. lutea* populations was relatively high but decreased from south to north with the lowest in Daya Bay. Numerous species originate close to the equator, a region with a stable SST and sunny environment (Roy et al., 1998; Jansson et al., 2013). Fossil and phylogenetic evidence support the latitudinal diversity gradient (Roy et al., 1998; Jansson et al., 2013). Additionally, the monthly average SST decreased significantly with increasing latitude (**Figure 4**). Thus, SST differences across latitude might strongly influence the genetic diversity gradient of *P. lutea* in the SCS.

The adaptability of organisms is mainly influenced by reproduction patterns and genetic diversity (Bernhardt and Leslie, 2013; Wu et al., 2021). Our findings suggest that sexual reproduction and rich genetic diversity will benefit the adaptability of *P. lutea* in tropical areas. First, corals add new alleles to the gene pool through sexual recombination, increasing within-population genotypic diversity

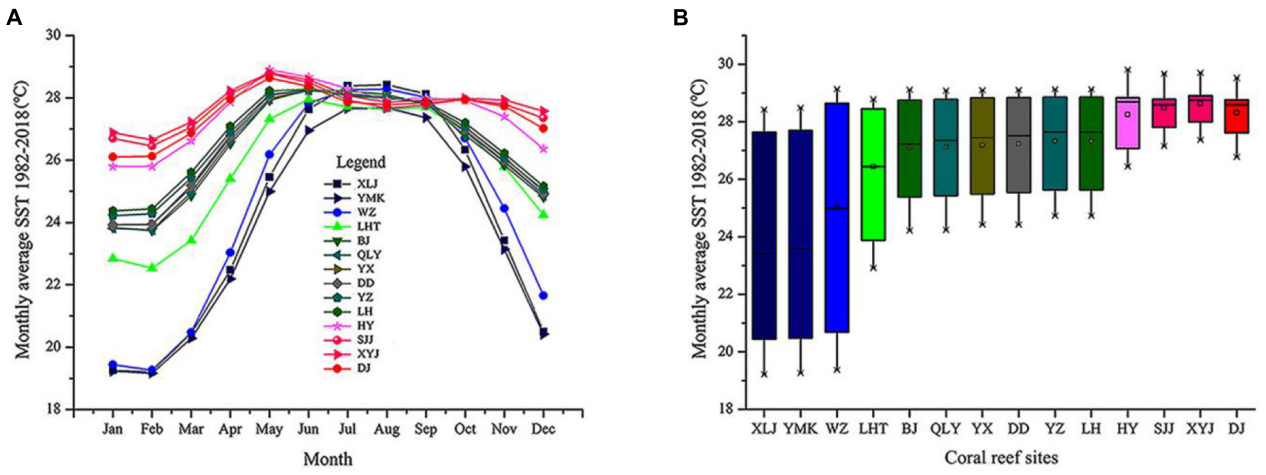

FIGURE 4 | Monthly average sea surface temperatures (SST) from 1982 to 2018 for 14 coral reef sites in the South China Sea. **(A)** Monthly averages and standard deviations of SST at all coral sites. **(B)** Box plots indicating annual mean, minimum, and maximum seasonal SST, grouped by coral reef sites.

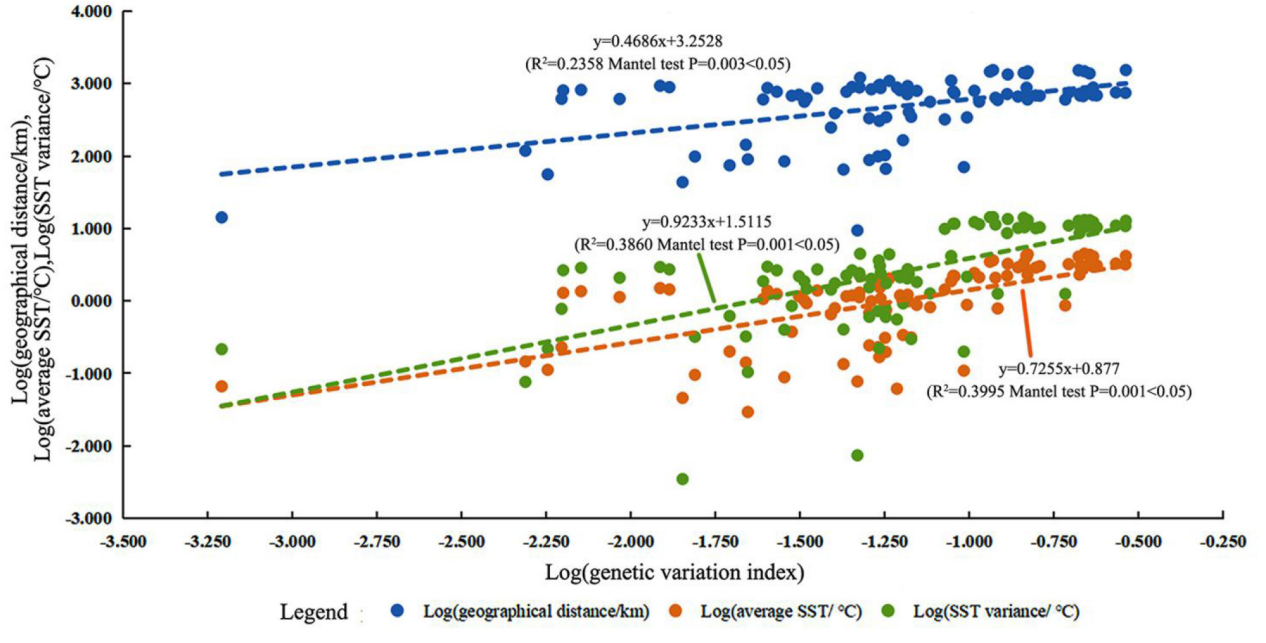

FIGURE 5 | Relationship between genetic variation (pairwise Φ_{PT} value) and geographic distance (marked in blue), average sea surface temperature (SST) (marked in orange), and SST variation (marked in green).

(Lasker and Coffroth, 1999; Hellberg and Taylor, 2002). Sexual reproduction allows coral larvae to inherit heat tolerance from both parents (Dixon et al., 2015; Kleypas et al., 2016). High genetic diversity enhances population sustainability over evolutionary time scales by providing sufficient alleles for adapting to future environmental change (Ayre and Hughes, 2004; Williams et al., 2014) through natural selection (Ficetola and Auré, 2011). Second, abundant genotypic diversity in populations is beneficial for improving fitness, stress response, and ecosystem function on an ecological time scale (Johnso et al., 2006; Hughes et al., 2008; Hughes and Stachowicz, 2009). Third, larval dispersal also increases genotypic diversity, a key factor affecting the population size and genetic diversity (Tay et al., 2015).

In Western Australia, *Pocillopora damicornis* populations in high-latitude areas exhibited reduced genetic diversity and restricted gene flow and were more vulnerable to global climate change due to their limited adaptability (Thomas et al., 2017). *Turbinaria peltata* populations had lower genetic diversity in the northern SCS, revealing their poor evolutionary potential (Wu et al., 2021). Compared to the subtropical *P. lutea* populations and the two corals above, tropical *P. lutea*

populations may have high adaptive potential. Furthermore, compared to *Acropora* corals in Luhuitou, *P. lutea* in the SCS had high zooxanthellae density. The primary dominant symbiont in *P. lutea* (C15) exhibits high thermal tolerance (LaJeunesse et al., 2010; Chen et al., 2019). Therefore, *P. lutea* was less susceptible to bleaching and had a high tolerance to thermal stress event (Xu et al., 2017; Qin et al., 2019). Indeed, massive corals, including *P. lutea,* have experienced the slowest decline in the face of rapidly degrading global coral reefs (Huang et al., 2021). Taken together, our results suggest that tropical *P. lutea* populations may be highly adaptable.

Spatial Sea Surface Temperature Variation Across Latitudes Affected *Porites lutea* Genetic Structure in the South China Sea

We detected significant genetic divergence between subtropical (particularly in Daya Bay) and tropical *P. lutea* populations, as confirmed by the pairwise F_{ST} values (**Supplementary Table 3**). Compared with the results obtained by Huang et al. (2018), we observed a stronger latitude-based difference in the genetic structure and identified a latitudinal structure gradient. Microsatellites are widely dispersed in eukaryotic genomes and have higher levels of polymorphism than nuclear markers (Baums et al., 2005; Wang et al., 2009). Thus, the genetic structure can be identified to a finer resolution using microsatellites (Ridgway and Gates, 2006). In this study, the SST dominated the genetic structure of *P. lutea* in the SCS. Meanwhile, geographic distance had a relatively small impact on genetic structure. Similarly, the SST had a closer connection with genetic structure than geographical isolation of *Turbinaria peltata* in the north SCS (Wu et al., 2021).

Temperature is a crucial environmental factor determining the distribution of poikilothermic invertebrates over latitudinal clines, particularly in corals (Parmesan, 2006; Li et al., 2009; Obura, 2012; Howells et al., 2014). The connectivity and structure of coral populations are directly tied to temperature across different latitudes (Hoegh-Guldberg and Pearse, 1995; Levin, 2006; Bradbury et al., 2008). Temperature is the dominant influence on the rates of fundamental biochemical processes regulating development and survival (O'Connor et al., 2007). Ecological simulation experiments indicated that bleaching would occur if *P. lutea* was exposed to 14°C for 3 days (Li et al., 2009). Furthermore, temperature can alter metabolic rates in individuals, thereby influencing the rates of genetic variation and evolution among populations (Rohde, 1992; Rosenzweig, 1995; Allen et al., 2002; O'Connor et al., 2007). Finally, the SST impacts the spawning period of coral and probably influences gene exchange by driving asynchronous spawning across latitudes (Hanafy et al., 2010; Howells et al., 2014). The coral spawning period in the Gulf of Oman (northwest Indian Ocean) is directly associated with latitudinal variation and is likely influenced by the SST and timing of lunar cycles (Howells et al., 2014). The spawning period at low latitudes is generally earlier than that at higher latitudes, which may be influenced by the SST (Wei et al., 2020). In addition, studies demonstrated that the same coral genus had different gamete development cycles and spawning period in different areas (Baird et al., 2009; Yang, 2013). Hence, the spawning period of *P. lutea* at different latitudes might be different. The lack of synchronization in spawning periods possible reduces larval recruitment between coral reef sites, ultimately affecting gene exchange and genotype combination between corals at different latitudes.

Geographic distance was also significantly positively correlated with genetic differentiation. The broadcast spawning strategy and high environmental tolerance help species to disperse over long distances (Polato et al., 2010; Baums et al., 2012). The *P. lutea* populations in this study were across 13° of latitude, with the largest distance between them being 1,500 km. However, there are no consistent ocean currents in the SCS to transport corals over long distances in a certain direction (Huang et al., 1992; Van der Ven et al., 2016). Therefore, geographic distance had a relatively small impact on genetic structure. Geographic distance was reported to affect the genetic structure of *T. peltata* in the northern SCS (Wu et al., 2021). Taken together, our results clearly indicate that latitudinal differences in the SST and geographic distance were the main factors influencing the genetic structure of *P. lutea* in the SCS.

Management Implications Under Climate Change

As discussed, tropical *P. lutea* populations may have a greater adaptability to anthropogenic disturbance and environmental change because of their high genetic diversity and sexual reproduction. In recent years, although tropical *P. lutea* populations may be more adaptable, coral bleaching has occurred frequently, and coral reef coverage has declined due to external environmental changes. We found that tropical *P. lutea* grew well in the SCS and had high coverage. However, high-latitude areas are regarded as a refuge for tropical coral species (Riegl, 2003; Beger et al., 2014; Thomas et al., 2017). As the global temperature rises, the SST of subtropical areas has become increasingly suitable for coral growth, and thus corals may shift their distributional ranges toward higher latitudes (Halfar et al., 2005; Chen et al., 2009). For instance, two tropical coral species have been expanding toward higher latitudes with a diffusion of 14 km/a since the 1930s during a century of global warming (Yamano et al., 2011). However, other factors may prevent the consolidation of subtropical regions as new coral refuges. Severe anthropogenic activities deteriorated the marine ecological environment in the subtropics. In particular, subtropical coral reefs of the SCS are mainly distributed along the coast, making them more susceptible to anthropogenic impact. In general, coral reefs are among the most vulnerable and easily disturbed ecosystems and need to be protected systematically. Firstly, the anthropogenic impact in relatively high latitudes should be removed to protect potential further refuges. Secondly, measures to enhance genetic potential and adaptability should be taken in the SCS. Thirdly, stress-tolerant species or populations are needed for artificial transplantation to help natural populations cope with global change.

CONCLUSION

Tropical *P. lutea* populations in the SCS show greater evolutionary potential because they exhibit abundant genetic diversity and sexual reproduction. The genetic diversity of *P. lutea* generally changes with increasing latitude, a gradient that appears largely due to latitudinal SST variation. Subtropical coral populations have lower genetic diversity, and thus, relatively poor genetic resilience in response to low average SST and human activities. Analyses of genetic variation, F_{ST}, and genetic clusters revealed that subtropical *P. lutea* populations are genetically distinct from the tropical populations. SST is likely to be a key factor affecting these genetic differences. Thus, protection measures could be considered to enhance coral cover at reef sites that would likely act as refuges of subtropical areas in the future, such as removing anthropogenic impacts (port construction, overfishing, and city development) and promoting artificial transplantation. Our research provides insight into coral genetics and scientific guidance and a theoretical basis for the protection and restoration of coral reefs.

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.

AUTHOR CONTRIBUTIONS

KY and WH designed the research. BC, XH, and ZQ contributed study materials. YL and ML performed the experiments. YL analyzed the data and generated all images. YL and WH wrote the manuscript. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.791149/full#supplementary-material

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