Received: 20 October 2022 | Revised: 17 January 2023 | Accepted: 19 January 2023

DOI: 10.1002/eap.2825

ARTICLE

ECOLOGICAL APPLICATIONS
ECOLOGICAL SOCIETY OF AMERICA

Parasites indicate trophic complexity and faunal succession in restored oyster reefs over a 22-year period

Christopher S. Moore[1] | **Christopher J. Baillie**[1] | **Emily A. Edmonds**[1] | **Rachel K. Gittman**[1,2] | **April M. H. Blakeslee**[1]

[1]Biology Department, East Carolina University, Greenville, North Carolina, USA

[2]Coastal Studies Institute, East Carolina University, Wanchese, North Carolina, USA

Correspondence
Christopher S. Moore
Email: christophermoore@fau.edu

Present addresses
Christopher S. Moore, Harbor Branch Oceanographic Institute, Florida Atlantic University, Fort Pierce, Florida, USA; Christopher J. Baillie, Eastern North Carolina Sentinel Landscape Partnership; North Carolina Coastal Federation, Newport, North Carolina, USA; and Emily A. Edmonds, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA.

Funding information
Garden Club of America, Grant/Award Number: Fellowship in Ecological Restoration; National Oceanic and Atmospheric Administration, Grant/Award Number: A19-0052-001; North Carolina Department of Environmental Quality, Grant/Award Number: NA15NOS4190091; North Carolina Collaboratory, Grant/Award Number: 20170 12593 221010 KOAST; U.S. Fish and Wildlife Service, Grant/Award Number: F19AP 00230

Handling Editor: Nguyen Esmeralda López Lozano

Abstract

Foundation species like the eastern oyster (*Crassostrea virginica*) create complex habitats for organisms across multiple trophic levels. Historic declines in oyster abundance have prompted decades of restoration efforts. However, it remains unclear how long it takes for restored reefs to resemble the trophic complexity of natural reefs. We used a space-for-time approach to examine community succession of restored reefs ranging in age from 3 to 22 years old in coastal North Carolina, surveying both free-living taxa and parasite communities and comparing them to natural reefs that are decades old. Trophically transmitted parasites can serve as valuable biodiversity surrogates, sometimes providing greater information about a system or question than their free-living counterparts. We found that the diversity of free-living taxa was highly variable and did not differ among new (<10 years), old (20 years), and natural reefs. Conversely, parasite diversity increased with elapsed time after restoration, and parasite communities in older restored reefs resembled those found in natural reefs. Our study also revealed that oyster toadfish (*Opsanus tau*) act as a key host species capable of facilitating parasite transmission and trophic ascent in oyster reef food webs. Overall, our results suggest that trophic complexity in restored oyster reefs requires at least 8 years to resemble that found in natural reefs. This work adds to a growing body of evidence demonstrating how parasites can serve as biodiversity surrogates, proxies for the presence of additional taxa that are often difficult or impractical to sample. Given the multiplicity of links formed with their hosts, parasites offer a powerful tool for quantifying diversity and trophic complexity in environmental monitoring studies.

KEYWORDS
community assembly, ecological restoration, environmental monitoring, oyster reefs, parasite diversity, trophic complexity

INTRODUCTION

Ecological restoration can serve as a tool for testing questions related to community assembly, as practitioners often seek to restore whole ecosystems using foundation species. In coastal environments, restoration efforts often focus on recreating structurally complex habitat using biogenic or "habitat-forming" organisms like seagrasses, mangroves, and oysters (Bayraktarov et al., 2020). These and other foundation species have suffered major declines (Altieri & von de Koppel, 2014), prompting calls by the United Nations for the current decadal focus on ecological restoration (UN, 2021). Oyster reefs are among the most degraded coastal habitats globally (Beck et al., 2011) and have been the focus of restoration efforts for over 50 years (Hernández et al., 2018). However, despite significant investment in restoration, only an estimated 4.5% of reef area has been recreated relative to historical baselines (Hernández et al., 2018). While these gains are important, it remains difficult to assess the long-term persistence and ecological function of restored oyster reefs given the short-term (1–3 years) monitoring typical of many projects (Baggett et al., 2015). Further, from a practical standpoint, it can be challenging to quantify biodiversity and trophic complexity of oyster reef-associated communities (La Peyre et al., 2019). Conventional sampling methods (i.e., nets, traps, trawls) all have species-specific limitations and efficiencies (Stone & Brown, 2018), which complicates efforts to obtain a holistic picture of the diverse invertebrate and vertebrate communities associated with reef habitat (La Peyre et al., 2019).

Rather than monitor entire ecosystems, ecologists often survey groups of "surrogate species" that are representative of their associated community (Wiens et al., 2008). Typically, researchers use free-living species as proxies for other taxa (Lindenmayer et al., 2015). However, parasites can serve as better indicators of underlying complexity because they serve as cross-taxon surrogates (Caro, 2010) for the presence of multiple invertebrate and vertebrate hosts (Hechinger & Lafferty, 2005; Hechinger et al., 2007). While free-living surrogates are often assumed to represent their target organisms through a shared preference for microhabitat or functional group status (Cushman et al., 2009), trophically transmitted parasites require specific hosts from different taxonomic groups spanning multiple trophic levels, making them excellent surrogates for overall community diversity. Moreover, host diversity is often strongly correlated with parasite diversity (Kamiya et al., 2014; Wood & Johnson, 2016), and evidence suggests that the abundance of trophically transmitted parasites is positively associated with predator–prey interactions and the density of intermediate host taxa in food webs (Rossiter & Sukhdeo, 2014). Parasites thus provide additional insight into trophic complexity, as increased parasite diversity correlates with greater overall biodiversity across taxonomic groups (Hechinger & Lafferty, 2005; Moore et al., 2020).

In our study, we examined a time series of restored oyster reefs constructed 3, 8, 19, and 22 years prior to our sampling (i.e., 2016, 2011, 2000, and 1997) as well as comparable natural reefs within the same estuarine research reserve complex. Space-for-time substitution is often used in ecological studies to infer how temporal processes like succession and community assembly correlate with different aged sites (reviewed in Wogan & Wang, 2017). This method is ideal for working with time-series data from multiple sites when inferences are made regarding ecological processes operating at the same spatial scale (Damgaard, 2019). We hypothesized that older restored reefs would exhibit greater host–parasite diversity compared to more recently restored reefs that would have accrued fewer trophic interactions. Reef age is clearly important for mediating community assembly, as multiple studies have documented faunal succession in restored reef–associated communities (La Peyre et al., 2019). However, environmental and methodological variation among studies often precludes drawing firm conclusions about the role of time itself in the succession process. In our study, we addressed these challenges using a surrogate species approach for determining trophic complexity by sampling multiple sites of different ages in the same comparative environment.

METHODS

Study site and experimental design

Our study was conducted in the Middle Marsh portion of the Rachel Carson Estuarine Research Reserve (34°41′32″ N, 76°37′16″ W), located along North Carolina's central coast (Figure 1). Middle Marsh is polyhaline and experiences a semidiurnal tidal exchange of ±0.9 m (Ridge et al., 2017). Over the past 22 years, researchers have built nearly 100 intertidal oyster reefs in the reserve, approximately 50 of which remain. These restored reefs vary in proximity to other landscape features such as sandflats, salt marshes, seagrass beds, and natural oyster reefs (Ziegler et al., 2018). Importantly, all reefs in our system are in close physical proximity (<1 km), ensuring relatively similar environmental conditions, and all have a clearly defined footprint. Restored reefs were constructed using loose, weathered oyster shell shaped into rectangular reefs (~3 m wide × 5 m long × 0.30 m tall) using hand tools and a modified oyster dredge. Additional details on site placement and methods can be found in Grabowski et al. (2005) (1997/2000 reefs) and Fodrie et al. (2014)

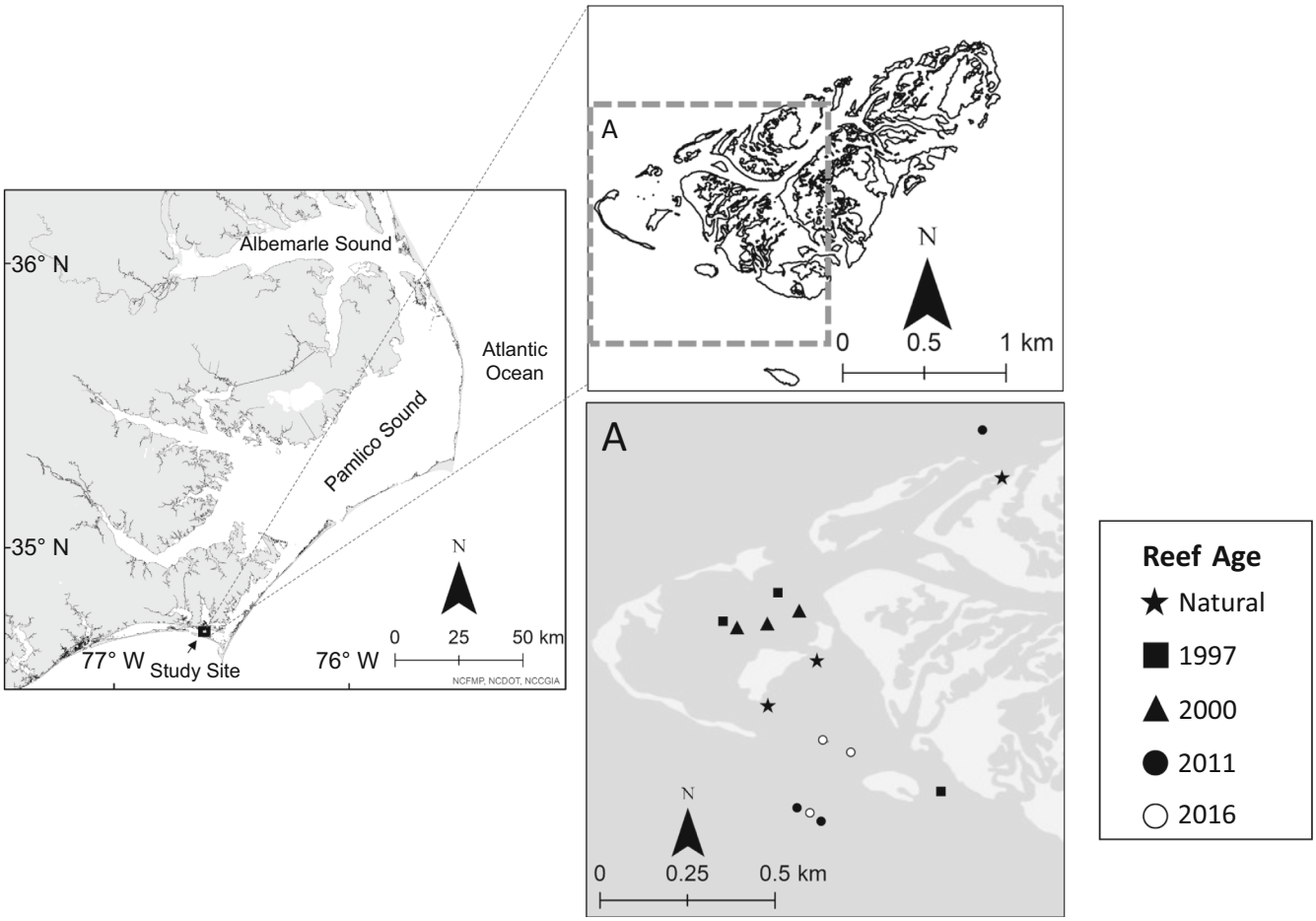

FIGURE 1 Map showing study site location along central North Carolina coast and enhanced view of Middle Marsh and project area (a).

(2011/2016 reefs). We sampled a subset of $n = 3$ reefs each restored in 1997, 2000, 2011, and 2016, along with $n = 3$ natural reefs that likely formed within the last 200 years (Ridge et al., 2017), for a total of 15 reefs.

Sampling for oyster reef fauna

Reef-resident fauna were sampled using passive collectors: small plastic milk crates (19 × 22 × 16 cm) filled with approximately 1.7 kg of autoclaved oyster shell. Collecting units such as these provide a standardized volume of habitat for use in monitoring patch reefs of different sizes (Moore et al., 2020). Two crates were deployed at each replicate treatment making for a total of $n = 30$ crates (2 crates × 3 replicates × 5 treatments). Crates were deployed in the shallow subtidal zone approximately 1 m from the base of each reef to avoid desiccation at low tide. Plastic crates were initially deployed in June 2019 and sampled approximately every 4 weeks until late October, after which all equipment was removed. Because of the abundance of crustaceans in our system, all shrimps and crabs were collected from only one crate at each reef, which was selected randomly. Resident fishes were less abundant and sampled from both crates. All organisms were placed in labeled bags and identified to species or genus level. We also used unbaited minnow traps (0.635 cm-mesh; 2.54 cm openings) to sample transient fauna during periods of inundation. At midrising tide 1 day prior to sampling, two minnow traps were deployed on opposite ends of each reef. Twenty-four hours later, the contents of both traps were checked along with those of the plastic crates. All fish sampled from the minnow traps were identified to species and released. During each sampling event, salinity, temperature (°C), and dissolved oxygen (mg/L) were measured using a handheld YSI (Pro-ODO).

Host dissection and parasite identification

Xanthid crabs and small benthic fishes were dissected for parasites since these are reef-resident organisms and known hosts for parasites in our system (Moore et al., 2020). Xanthid crab hosts included the stone crab *Menippe mercenaria* and four species of panopeid

mud crabs: *Rhithropanopeus harrisii*, *Eurypanopeus depressus*, *Panopeus herbstii*, and *Dyspanopeus sayii*. Fish hosts included gobies *Ctenogobius boleosoma*, *Gobiosoma ginsburgi*; blennies *Chasmodes bosquianus*, *Hypsoblennius hentz*, *Hypleurochilus geminatus*, *Parablennius marmoreus*; and oyster toadfish *Opsanus tau*. In general, the crab and fish species selected for dissection display strong site fidelity to specific reefs (Harding et al., 2019; Toscano et al., 2014). In all hosts, we identified common trophically transmitted endoparasites, including nematodes, digenetic trematodes, acanthocephalans, and cestodes. These macroparasite taxa develop within their hosts and require multiple invertebrate and vertebrate hosts for lifecycle completion (Poulin, 2007). We did not sample ectoparasites like monogeneans and copepods because these organisms are easily dislodged in the process of collection and transport. For panopeid mud crabs only, we also quantified *Loxothylacus panopaei*, an obligate rhizocephalan parasite of mud crabs with a direct life cycle that can exhibit moderate to high infection prevalence in the region (Blakeslee et al., 2021).

Crabs were measured (carapace width, mm), sexed, and dissected by separating the upper and lower carapace. Tissue squashes of hepatopancreas and gonad were scanned for parasites at low power (4–10×) using a compound microscope (Zeiss AxioScope A1). Fish were measured (total length, in millimeters), sexed, and dissected by removing the entire gastrointestinal tract (stomach, liver, gallbladder, spleen, intestine) and scanning for parasites at low power. The gut cavity of each fish was then rinsed and the wash examined for parasites that had been dislodged during dissection. Lastly, the head, body, and fins were checked for subcutaneous trematode cysts by viewing each fish at low power under a stereomicroscope (Zeiss Stemi 508). Only xanthid crabs ≥5 mm and benthic fishes ≥20 mm were dissected for parasites because the macroparasites in our system primarily infect adult individuals (Moore et al., 2020). All parasites were identified using standard protocols and keys (e.g., Yamaguti, 1971). Although smaller mud crabs and fish were not dissected for parasites, they were still included as part of our free-living diversity analyses. Field collections were authorized by the North Carolina Division of Marine Fisheries (Scientific or Educational Permit 706671) and by the North Carolina Coastal Reserve (Permit 13-2019). Animal husbandry and dissection protocols were approved by East Carolina University's (ECU) Institutional Animal Care and Use Committee (Animal Use Protocol no. D346, no. D358).

Oyster reef habitat parameters

We assessed habitat complexity in July–August 2019 by measuring multiple structural features in all reefs. In July, reef area (in square meters), reef height (in meters), maximum reef height (in meters), fringe elevation (in meters), crest elevation (in meters), and crest peak elevation (in meters) were quantified using a Trimble R10 Global Navigation Satellite System (<1.5-cm vertical precision). In August, two 0.0625-m^2 quadrats, one from the crest and one from the fringing slope of each reef, were excavated to a depth of 10 cm. From these quadrats, the following metrics were collected: oyster shell dead total weight (in grams), live oyster count (n), live oyster weight (in grams), and live oyster length (in millimeters). The total count of all live oysters >20 mm and the length of up to 50 randomly selected live oysters were quantified for each sample. Correlation analyses revealed that most habitat parameters were either moderately or strongly correlated (coefficient ±0.3). As a result, we only compared reef area, reef height, and live oyster count among reefs because these variables were only weakly correlated (±0.2). Moreover, all three variables were identified by Baggett et al. (2015) as key performance indicators of functioning oyster reefs.

Parasite diversity and trophic complexity

We created food webs depicting how parasite diversity differed in a focal host species, the oyster toadfish, which was the dominant fish-host for parasites in our system. An organism's location within a food web is an important predictor of its parasite diversity. Fish species in the middle of food webs consume a variety of invertebrate and vertebrate prey, often harboring the greatest number of endoparasites (Marcogoliese, 2002; Poulin & Leung, 2011). Dietary studies have revealed that adult toadfish occupy an intermediate trophic level of 3.8 ± 0.04 (Froese & Pauly, 2010). In our sampling, we captured juveniles and young adults (≤150 mm). We categorized toadfish as either juveniles (<100 mm) or adults (>100 mm) according to data from Wilson et al. (1982).

Parasite data from infected toadfish were used to construct food webs for new (<10 years), old (20 years), and natural reefs by extrapolating the additional taxa required by these parasites for life cycle completion based on known toadfish predator–prey relationships (Linton, 1905; Moore et al., 2020; Schwartz & Dutcher, 1963; Wilson et al., 1982). In the natural reefs, we documented a single toadfish infected with an adult acanthocephalan. Although we excluded this one observation from our multivariate analysis, we included it as part of our food web analysis. Taxa located in Trophic levels 1, 2, and 3 are known hosts for trophically transmitted parasites (Moore et al., 2020). They are also common prey items of toadfish based on dietary studies (Wilson et al., 1982). Taxa located at Trophic

level 4 are putative hosts based on published data (Linton, 1905) and their abundance in our system (Moore et al., 2020).

Statistical analyses

To determine the drivers of diversity as a function of reef age, we analyzed the following data sets: free-living crustacean host taxa (hereafter: free-living crustacean taxa), crustacean parasite taxa, free-living finfish host taxa (hereafter: free-living fish taxa) and fish parasite taxa. For the free-living fish taxa, we combined data from the minnow traps and plastic collectors to create a single data set of transient and reef-resident fishes. Parametric statistical tests were used when the data met the assumptions of a Gaussian distribution. Nonparametric tests were used when the data did not meet the assumptions of normality and could not be transformed. Abiotic data (salinity, temperature, dissolved oxygen) were not included in these statistical analyses because they were not collected at each site and thus could not be used in comparisons among reefs (Appendix S1: Table S1).

We created box plots depicting taxa richness as a function of time elapsed since restoration. For each response variable, we tested for significant differences between reefs (2016, 2011, 2000, 1997, natural) using one-way ANOVAs (free-living taxa) and nonparametric Kruskal–Wallis tests (parasite taxa). Post hoc testing was performed using Tukey's post hoc (free-living taxa) and Wilcoxon rank-sum tests adjusted for multiple pairwise comparisons (parasite taxa). Effect sizes were calculated using Cohen's *D* (free-living taxa) and Wilcoxon effect size tests (parasite taxa) to determine the magnitude of difference between natural reefs and restored reefs of each age class. Welch's *t*-tests for unequal variance were used to compare the overall magnitude of effect between crustacean taxa versus parasites and fish taxa versus parasites. Shannon–Weiner diversity values were calculated using the *vegan* package (Oksanen et al., 2020). For both crustaceans and fish, we also evaluated the strength of the correlation between host and parasite richness as a function of reef age across new (<10 years), old (20 years), and natural reefs. For the response variables in these plots, the residuals were normally distributed, and Pearson's correlation coefficients were fitted to the data. For our analysis of habitat, Kruskal–Wallis and Wilcoxon rank-sum post hoc tests were used to test for differences in reef area, reef height, and the number of live oysters. Univariate analyses were conducted in R (version 4.0.3) using R core functionality and the rstatix package (Alboukadel, 2020).

We used nonmetric multidimensional scaling (nMDS) ordinations (Field et al., 1982) based on Bray–Curtis dissimilarity matrices in PRIMER version 7 to visualize changes in host–parasite abundance during our project. The fish parasite data contained a single observation of an adult acanthocephalan parasite, which was removed prior to analysis. All abundance data were then fourth-root transformed. Pearson correlation coefficient taxa overlays were established for each nMDS plot at a threshold ≥0.35. In addition, a permutational multivariate ANOVA (PERMANOVA) was conducted to examine whether host/parasite abundance differed between new (<10 years), old (20 years), and natural reefs. We used a nested design with sampling event as a random effect within reef age to avoid pseudo-replication. Pairwise comparisons were made among the data in different age groups via the unrestricted permutation of abundance data. Based on these results, a similarity percentage analysis (SIMPER) was performed on the fish parasite abundance data to determine which parasite taxa were responsible for observed differences between reef age groups.

Finally, we sought to understand the drivers of oyster toadfish abundance in our system, as toadfish were the species of fish most often parasitized. Generalized linear mixed models were fit to the data using a negative binomial distribution via the model-building package glmmTMB (Brooks et al., 2017). The following reef habitat parameters were included as fixed effects: reef age (new, old, natural), reef area (in square meters), and reef height (in meters). Live oyster count was excluded because there were no differences among treatments. We also included two landscape-level predictors: proximity to the nearest marsh habitat (in meters) and the extent of south-facing fetch (in meters) (i.e., the distance wind blows without obstruction). The former has been shown to enhance densities of invertebrate taxa in our system (Ziegler et al., 2018), while the latter acted as a proxy for seasonally driven wave energy during our project. We controlled for pseudo-replication in our random effects by nesting site within treatment (natural, 1997, 2000, 2011, 2016). All models incorporating area and age were fit using an interaction term because these predictors were assumed to covary. Reef area and marsh proximity were strongly correlated, and these terms were binned into separate models. Where multiple numerical predictors were evaluated in the same model, variables were centered with a mean of zero to facilitate comparison (McElreath, 2016). Model diagnostics and fit were assessed using the packages DHARMa (Hartig, 2022) and performance (Lüdecke et al., 2021). Additional criteria used to evaluate model performance are provided in the supplements (Appendix S2: Section S1). Models were ranked using Akaike's information criterion adjusted for small sample size (AIC_c) (Akaike, 1974), compared via the AICcmodavg package (Mazerolle, 2020), and interpreted using multimodel inference (Burnham & Anderson, 2004).

Likelihood ratio tests were used to evaluate the predictive ability of top-performing models relative to a null model without any regression parameters. In each case, candidate models were tested against a reduced model with only the response variable and an intercept term.

RESULTS

Comparison of free-living and parasite diversity in natural and restored reefs

There were no differences in free-living crustacean and fish taxa richness or diversity as a function of restored reef age (Figure 2A,B; Appendix S3: Figure S1A,B). However, fish parasite richness in the natural reefs was greater than richness in the 2016 reefs (Figure 2D, $\chi^2 = 12.5$, df $= 4$, $p = 0.02$). Shannon diversity of fish parasites demonstrated a marginally significant relationship with time elapsed since restoration, although post hoc testing revealed no significant differences between treatments (Appendix S3: Figure S1D, $\chi^2 = 8.6$, df $= 4$, $p = 0.07$). Measurements of effect size comparing the magnitude of difference in fish parasite richness between natural reefs and restored reefs demonstrated that data from the natural reefs were most dissimilar to reefs restored 3 years (3-natural, $p = 0.02$) and 8 years prior (8-natural, $p = 0.06$), with a large magnitude of effect in each case (Appendix S1: Table S2). On the other hand, data from the old reefs were not different from the natural reefs (19-natural, $p = 0.11$; 22-natural, $p = 0.30$), with only moderate and small effect sizes, respectively (Appendix S1: Table S2). Pooling the data separately for free-living taxa (crustaceans, fish) and parasite taxa (crustaceans, fish) showed that there was no obvious trend for the free-living taxa when comparing data from the natural reefs sequentially to data from the restored reefs (Figure 3A; Appendix S1: Table S2). However, the magnitude of difference in the parasite data between natural reefs and restored reefs decreased through time as parasite communities became more like those found in natural reefs (Figure 3B, Appendix S1: Table S2). Moreover, the effect of age on diversity was greater for crustacean parasites compared to free-living crustacean taxa ($t = -4.2$, df $= 14.5$, $p = 0.0008$) and for fish parasites compared to free-living fish taxa ($t = -4.5$, df $= 13.7$, $p = 0.0006$) (Appendix S3: Figure S2; Appendix S1: Table S2).

Correlation between host/parasite richness in new, old, and natural reefs

The positive relationship between free-living crustacean taxa and crustacean parasite richness strengthened with time (Figure 4A–C). While there was no relationship for new

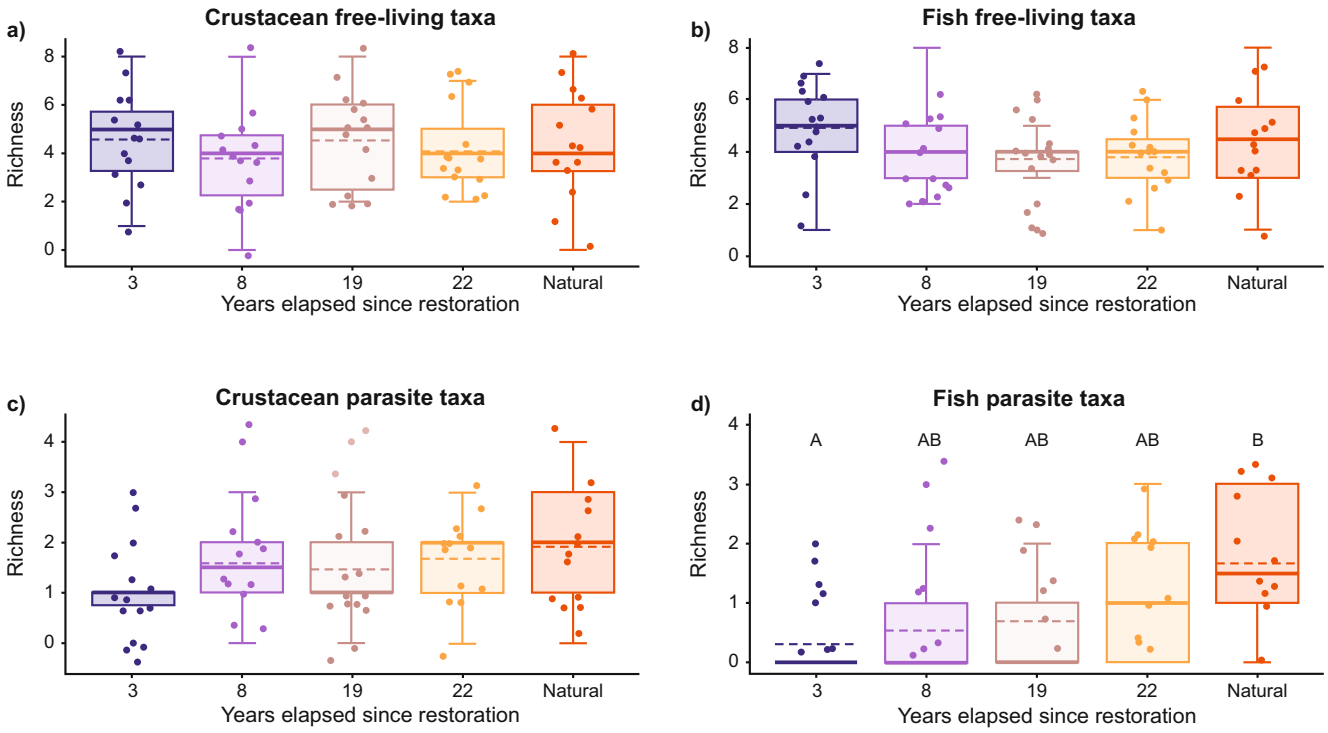

FIGURE 2 Free-living crustacean and fish taxa richness (a, b); crustacean and fish parasite taxa richness (c, d). Solid lines represent median of each treatment, dashed lines mean estimates. In panel (d), fish parasite richness in natural reefs was significantly greater than in reefs restored 3 years prior to our study; however, parasite richness in reefs restored 8, 19, or 22 years prior to our study did not differ from richness in natural reefs.

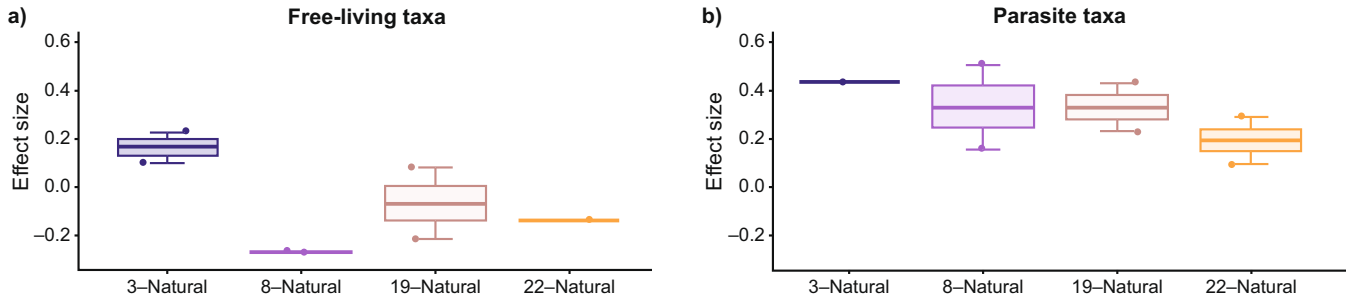

FIGURE 3 Comparison of effect size measurements between free-living taxa (a) and parasite taxa (b).

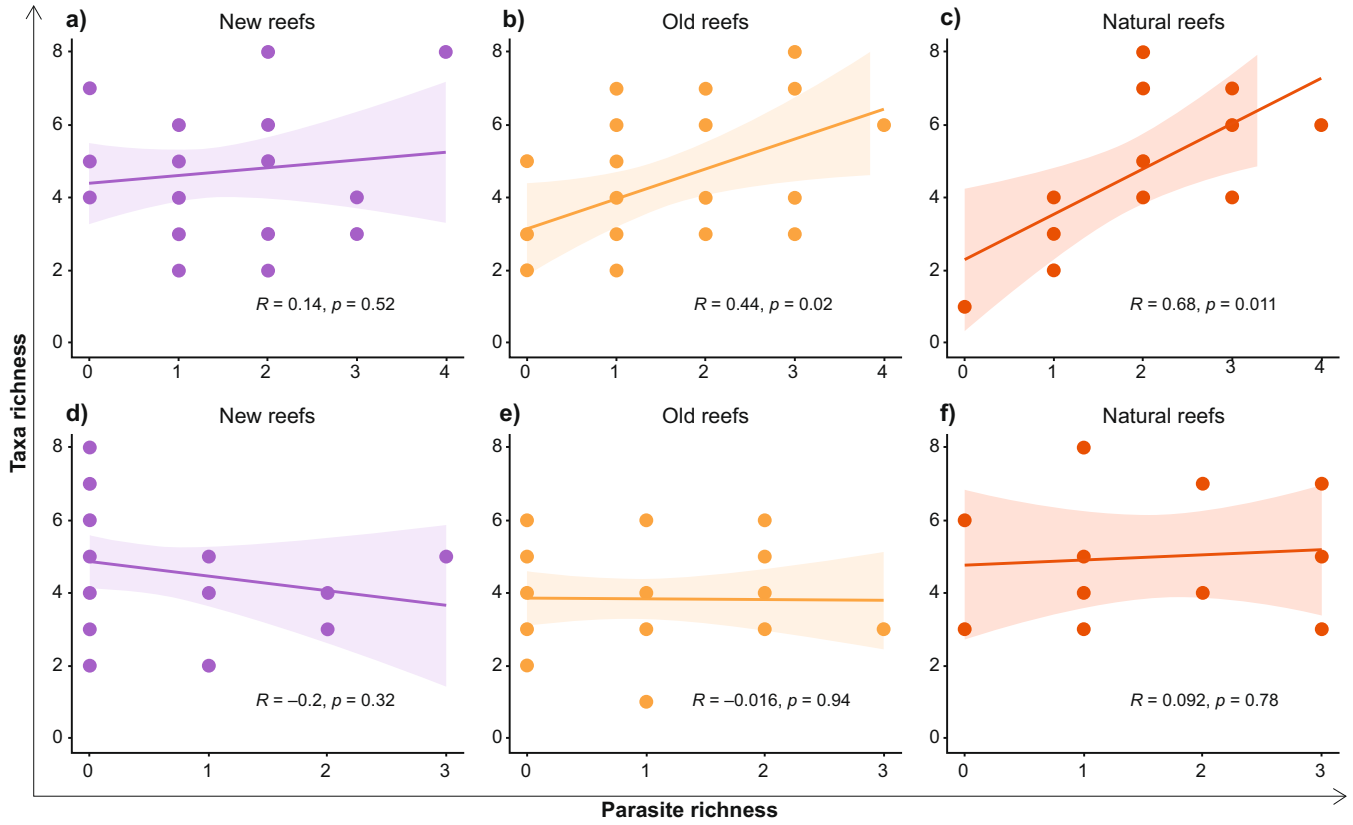

FIGURE 4 Correlation between free-living and parasite taxa richness in crustaceans (a–c) and fish (d–f) across new, old, and natural reefs. Shaded areas represent 95% CI for each fitted curve.

reefs ($R = 0.14$, $t = 0.66$, df $= 22$, $p = 0.52$), there was a positive relationship between crustacean host–parasite richness in old reefs ($R = 0.44$, $t = 2.49$, df $= 26$, $p = 0.02$), which strengthened in the natural reefs ($R = 0.68$, $t = 3.07$, df $= 11$, $p = 0.01$). There was no relationship between free-living fish and parasite richness (Figure 4D–F).

Changes in community abundance through time

nMDS analyses showed clear seasonal differences (summer to fall) in the relative abundance of free-living crustacean and fish taxa (Appendix S3: Figure S3A,B; Appendix S1: Table S3). However, there were no seasonal trends for crustacean and fish parasite abundance (Appendix S3: Figure S3C,D). A PERMANOVA with taxa abundance nested within sampling event showed that reef age (new [<10 years], old [20 years], natural) was an important predictor of fish parasite abundance (Appendix S1: Table S4, $p = 0.01$). The abundance of fish parasites was different between the natural and new reefs (Appendix S3: Table S4, $p = 0.005$) and between the natural and old reefs (Appendix S1: Table S4, $p = 0.050$). SIMPER analyses showed that these results were primarily driven by differences in the abundance of larval cestode (procercoid)

parasites (Appendix S1: Table S5). Complete species composition of all parasite taxa are available in Appendix S1: Table S5. Mean abundances (±SD) of all free-living taxa are available in Appendix S1: Table S6.

Oyster reef habitat complexity

Natural reefs (538 ± 293 m^2) were larger than old (78.2 ± 12.7 m^2) and new reefs (34.3 ± 13.9 m^2), while new reefs were smaller than old reefs (Appendix S3: Figure S4; Appendix S1: Table S7). Old reefs were taller (0.431 ± 0.0740 m) than natural (0.338 ± 0.0563 m) and new reefs (0.308 ± 0.0387 m), but there was no difference in height between natural and new reefs (Appendix S1: Table S7). The average number of live oysters was not different among new (130 ± 110), old (69 ± 42), and natural reefs (90 ± 109) (Appendix S1: Table S7).

Toadfish food web

Oyster toadfish comprised 72% of all parasitized fish (Appendix S1: Table S8). Overall, parasitized toadfish were most abundant in the natural reefs ($n = 32$), followed by the old ($n = 20$) and new reefs ($n = 4$) (Appendix S1: Table S9). The diversity of parasite taxa and life history stages increased across new, old, and natural reefs (Figure 5). New reefs had the lowest overall trophic complexity, as there were only two major parasite transmission pathways present (cestodes, nematodes), and juvenile toadfish were absent from these reefs (Appendix S1: Table S8). The old reefs featured juvenile and adult toadfish infected with cestodes, nematodes, and metacercarial-stage trematode parasites. The natural reefs had the greatest diversity of toadfish parasites (cestodes, nematodes, trematodes, acanthocephalans), some of which were final hosts for trematodes due to trophic transmission resulting from predation and cannibalism (Figure 5).

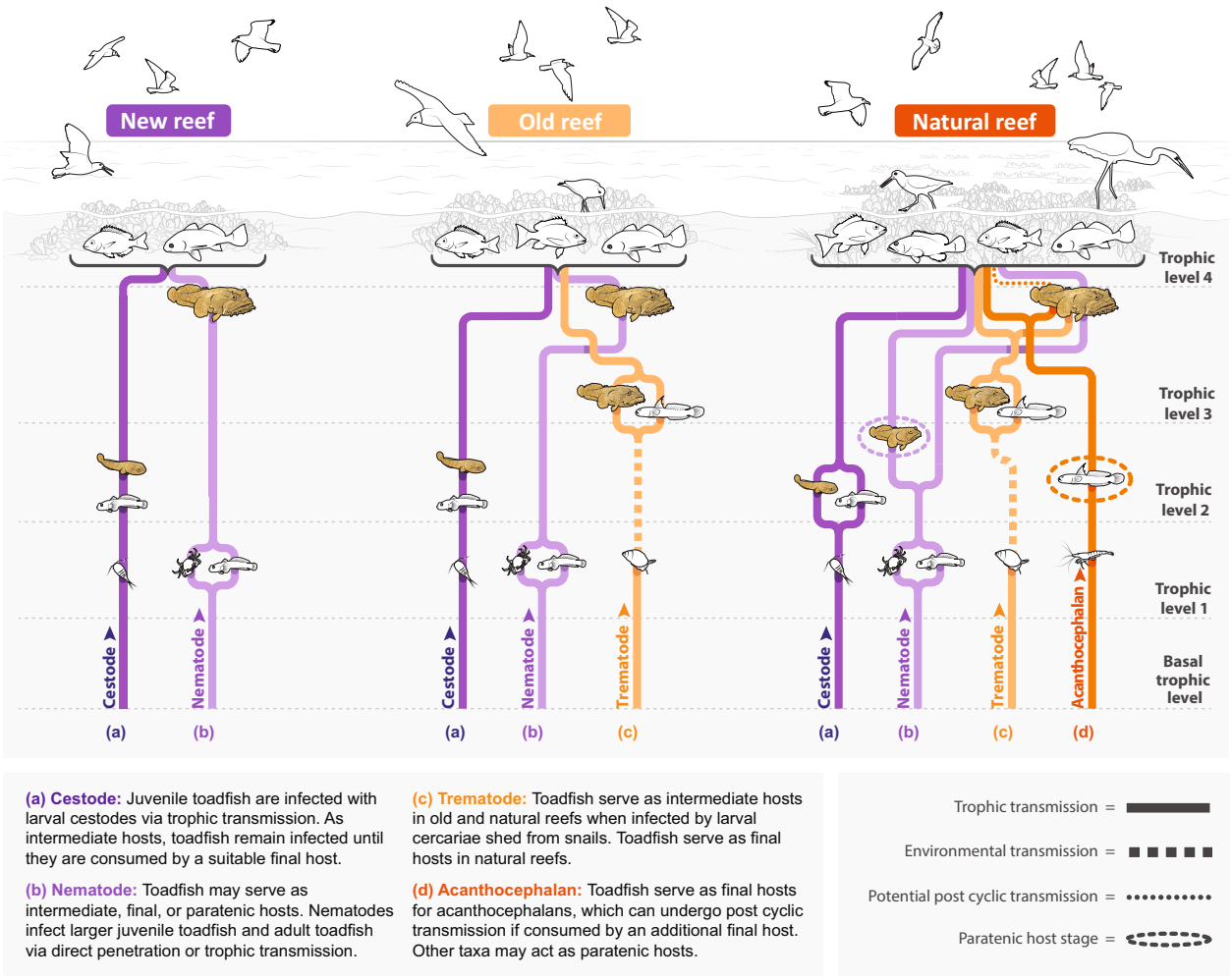

FIGURE 5 Food webs illustrating how parasite diversity in toadfish differs in new (<10 years), old (20 years), and natural reefs. Oyster toadfish are the only species depicted in solid form, since they are the focal organism in this figure, while other host taxa are depicted as silhouettes.

The two most parsimonious models for predicting toadfish abundance in our system were "reef age and marsh proximity" and "reef age and fetch" (Appendix S1: Tables S10 and S11). Full model outputs along with estimated 95% confidence intervals can be found in Appendix S1: Table S12. In the model incorporating fetch as a predictor, there was a negative relationship between toadfish abundance and new reefs ($z = -4.1$, $p = 3.5 \times 10^{-5}$) as well as old reefs ($z = -3.0$, $p = 0.003$), but natural reefs were positively associated with toadfish in our system ($z = 3.78$, $p = 1.6 \times 10^{-4}$) (Appendix S1: Table S12). Reef area was a component of the third most informative model, which identified a marginally significant positive interaction between area and toadfish abundance on new reefs ($z = 1.7$, $p = 0.09$), although the mean estimate for this term in the conditional model was highly uncertain (9.4 ± 5.6) (Appendix S1: Table S12). In all models, there was low variance in the random effects structure (Appendix S1: Table S12), suggesting little or no correlation between toadfish abundance and individual sample sites.

DISCUSSION

Ecological restoration projects can provide living laboratories for administering large-scale experiments under natural conditions. In marine systems, previous studies have concluded that free-living taxa readily recruit to restored oyster reefs 1–2 years after construction (Humphries et al., 2011; Moore et al., 2020) and that these habitats will have acquired some of the functional attributes of natural reefs (e.g., reef biomass, mesopredator abundance) after approximately 10 years (Smith et al., 2022). In other biogenic systems, studies have demonstrated that restored marine habitats increasingly resemble their natural counterparts over time. For example, Burt et al. (2011) compared patterns of community development in a time series of breakwater structures (1–31 years old), finding that artificial reef communities increasingly resembled, but did not replicate, those in natural coral reefs. These results were confirmed by Perkol-Finkel and Benayahu (2007) and Hill et al. (2021), who compared community structure on centuries-old artificial reefs to nearby natural reefs. Similarly, a synthesis of seagrass restoration projects (3–32 years old) showed that succession trajectories between restored and reference plots tended to converge over time, although there were distinct differences after >30 years between reference plots and transplant restorations (Rezek et al., 2019).

In our system, we used a space-for-time approach to analyze long-term successional patterns in oyster reef communities. We predicted that the diversity of parasites and hosts would increase after restoration, with older reefs approaching the diversity of natural reefs. In what follows, we discuss our results and how they advance the theoretical and applied framework for using multihost parasites as surrogate species in a restoration context.

Parasites indicate successional changes in oyster reef communities

We found that parasites could serve as better indicators of trophic complexity in oyster reefs compared to free-living taxa. While there was variability in the parasite data, mean estimates of richness and diversity of crustacean and fish parasites increased with elapsed time (Figure 2C,D; Appendix S3: Figure S1C,D). This trend was not apparent for free-living taxa (Figure 2A,B; Appendix S3: Figure S1A,B), whose relative abundance varied from month to month (Appendix S3: Figure S3A,B). These results suggest that conventional methods of sampling for oyster reef fauna (e.g., minnow traps, settlement trays/habitat collectors) using free-living organisms may be less effective at capturing long-term successional changes compared to sampling for surrogate taxa like trophically transmitted parasites, which represent whole communities rather than a subset of free-living organisms captured during field surveys. In addition, the overall magnitude of effect of reef age on taxa richness was greater for the parasites (Appendix S3: Figure S2; Appendix S1: Table S2), indicating a stronger relationship between parasite taxa richness and the age of restored reef habitat. For the parasite data only, we found a stepwise decrease in effect sizes from new–old–natural reefs (Figure 3B; Appendix S1: Table S2). This suggests that restored reefs increased in trophic complexity, becoming increasingly similar to natural reefs after two decades of elapsed time. While we sampled more free-living taxa than parasite taxa, it should be noted that effect size measurements are independent of sample size (Ho et al., 2019).

Although the parasite data were more informative overall, we identified important differences between parasites of crustaceans and parasites of fish when exploring the correlation between host and taxa richness as a function of reef age (Figure 4). Although there was no relationship between parasite and host taxa richness in the new (<10 years) reefs, there was a strong, positive relationship in the old (20 years) and natural reefs (Figure 4A–C), as these communities have had additional time to become established. Following restoration, our data suggest that it takes at least 8 years for crustacean parasite communities to resemble those found in natural

reefs (Appendix S1: Table S2). In contrast, we did not document this same relationship in fish (Figure 4D–F), likely due to the greater mobility and functional diversity (e.g., life history, habitat preferences) of fishes sampled compared to crustaceans (Appendix S1: Table S6). However, although we did not detect a strong host–parasite relationship collectively across all fish species, we found that oyster toadfish were a key focal host for parasites in our system.

Toadfish are a key host species in oyster reef food webs

Parasitism in food webs is a nonrandom process (Chen et al., 2008). Hosts with high parasite diversity tend to consume a wide variety of prey items and occupy network positions close to many types of prey (Marcogoliese, 2002). While trophically transmitted parasites require multiple hosts to complete life cycles (Huspeni & Lafferty, 2004), parasite diversity can be driven by a single host species or a few closely related species (Byers et al., 2008; Fenton et al., 2015), increasing the probability of successful transmission over evolutionary time if those hosts are central to the food web (Anderson & Sukhdeo, 2011). Thus, it is possible to map parasite transmission onto food webs since trophically transmitted parasites move along pathways in which key hosts act as trophic links (Poulin & Leung, 2011; Thompson et al., 2005). In our study, we identified toadfish as a key host species for mapping trophic interactions among reef-associated taxa using trophically transmitted parasites (Figure 5).

Past research has suggested that toadfish could mediate trophic cascades in oyster reef communities via predation (Grabowski et al., 2008; Grabowski & Kimbro, 2005). Toadfish occupy an intermediate position in food webs and often harbor higher numbers of endoparasites since they feed on a diverse array of invertebrate and small fish species that function as upstream hosts (Marcogoliese, 2002; Poulin & Leung, 2011). For example, in a study of three intertidal food webs, Chen et al. (2008) reported that species serving as intermediate hosts had more predators (mostly birds) and were incorporated into more food chains than those not serving as intermediate hosts. Indeed, parasites using intermediate hosts rely on predation to facilitate "trophic ascent" (i.e., Parker et al., 2015) to larger hosts at higher trophic levels where reproduction and dispersal occur (Esch & Fernandez, 1993).

Toadfish undergo ontogenetic shifts in diet (Wilson et al., 1982), which has implications for the upward incorporation of parasites in toadfish-driven food webs (Figure 5). For example, juvenile toadfish were primarily infected with larval cestodes, while larger adults had higher parasite diversity because of the wider range of prey items they consume (Appendix S1: Table S9). Multiple cestode taxa (e.g., *Diphyllobothrium*, *Triaenophorus*) use copepods as first intermediate hosts, followed by small fish (Pasternak et al., 1995). Smaller fish like juvenile toadfish are more likely to consume an infected copepod or other microcrustacean serving as a first intermediate host for cestode parasites (Poulin & Leung, 2011). While we documented larval cestode infections in adult toadfish from multiple reefs (Appendix S1: Table S9), these individuals were likely infected as juveniles because microcrustaceans would not be an important prey item of adult toadfish (Pasternak et al., 1995). Importantly, we did not document any mature cestode infections in toadfish, which could suggest that toadfish are not a final host for these parasites. It is also interesting that nearly all infected juvenile toadfish were sampled from the natural reefs (Appendix S1: Table S9). Indeed, the discrepancy in the abundance of larval cestode-infected fish was mostly responsible for driving differences in fish parasite abundance as a function of reef age (Appendix S1: Tables S4 and S5). The preponderance of cestode-infected toadfish in the natural reefs may imply that the process of trophic ascent for cestode parasites from first to final host is more efficient. Without an abundance of juvenile toadfish, cestodes may encounter a trophic "vacuum" (Parker et al., 2015) that would inhibit life cycle completion.

Larger toadfish are capable of consuming larger and more diverse prey such as small fishes, shrimps, and mud crabs (Linton, 1905; Wilson et al., 1982). While we identified multiple parasite taxa in adult toadfish (Appendix S1: Table S9), the diversity of parasites and life history strategies differed among reefs (Figure 5; Appendix S1: Table S9). For the most part, parasites like nematodes, trematodes, and acanthocephalans were absent from the new reefs. The lack of parasite diversity in fishes sampled from new reefs may suggest that the corresponding reef community is host-poor. However, low sample size may also account for these results, as we sampled fewer toadfish overall from the new reefs ($n = 4$) compared to the old ($n = 20$) and natural reefs ($n = 32$) (Appendix S1: Table S9). In both the old and natural reefs, multiple parasite taxa require toadfish as intermediate or paratenic hosts to fill the trophic transmission vacuum between first and final hosts (Benesh et al., 2014). Adult toadfish in these reefs were also parasitized by mature nematodes, trematodes, and acanthocephalans, consistent with the prediction that the diversity of adult parasites in a specific host would increase with increasing trophic level (Poulin & Leung, 2011). It is noteworthy that adult trematodes were absent from the old reefs, while toadfish from

the natural reefs were infected with both intermediate and adult-stage trematodes. Trematode life cycle completion requires trophic transmission, an outcome that is more favorable when there is a high abundance of intermediate hosts with overlapping niches (Choisy et al., 2003). This may suggest that the overall host community is not as diverse in the old reefs compared to the natural reefs, which also had an adult acanthocephalan infection. However, it is likely that we undersampled parasites like trematodes and acanthocephalans because our collection method was size-limited and biased toward smaller adult toadfish. Ultimately, our discussion of toadfish as key hosts in parasite food webs is meant to be illustrative rather than comprehensive. Comparing parasite diversity in toadfish sampled from new (<10 years), old (20 years), and natural reefs enabled us to depict the trophic connections represented by each toadfish–parasite interaction and infer the makeup of the overall host community.

Reef age and landscape-level factors influence trophic complexity

In addition to reef age, reef area may also be expected to influence host abundance and parasite diversity. Natural reefs were the largest reefs overall and harbored the greatest number of toadfish hosts (Appendix S3: Figure S4; Appendix S1: Table S9). The relationship between size and species richness is well known (i.e., Connor & McCoy, 1979), and thus greater reef size may contribute to greater species diversity. However, the old reefs were closer in size to the new reefs (Appendix S1: Table S7). Thus, if reef area were a better predictor of diversity, then parasite diversity in the old reefs should more closely resemble that of new reefs. Moreover, our models tested the interaction between area and age using habitat parameters that differed between reefs (e.g., area, age, height) or those that could explain patterns of toadfish abundance at the landscape scale (e.g., marsh proximity, extent of fetch) (Appendix S1: Table S10). In some cases, habitat structure can serve as a predictor of parasite transmission pathways (Rossiter & Sukhdeo, 2014). However, our results cannot solely be attributed to differences in the structural complexity of reef habitat, at least not for the parameters we measured. We found that neither area nor height was a component of the two most parsimonious models of toadfish abundance (Appendix S1: Table S10). Instead, a combination of "reef age and marsh proximity" and "reef age and fetch" accounted for most of the variation of toadfish in our system (Appendix S1: Table S10). In the model with fetch, there was a strong negative relationship between toadfish abundance and the new reefs ($z = -4.1$, $p = 3.5 \times 10^{-5}$), a pattern that weakened in the old reefs ($z = -3.0$, $p = 0.003$) and became positive in the natural reefs ($z = 3.78$, $p = 1.6 \times 10^{-4}$) (Appendix S1: Table S12). Areas with extensive fetch are prone to greater wave energy and tend to have less contiguous or complex habitat (Keller et al., 2019). Most of the new reefs are located on the more exposed southern end of Middle Marsh (Figure 1), which could explain the strong negative relationship with toadfish abundance. On the other hand, the old reefs are located in closer proximity to the natural reefs in a more sheltered part of Middle Marsh dominated by mudflats (Figure 1). Measurements of abundance and richness are notoriously scale-dependent; the directions of effects are often reversed at other scales of different magnitude (Chase et al., 2018). While fetch could inhibit the abundance of toadfish at the site level, fetch-prone areas could also serve as corridors at the landscape level for larger toadfish moving between habitat patches (e.g., mudflat, seagrass, salt marsh). In general, the extent of habitat linkages is an important predictor of species richness and community composition in marine environments (Gain et al., 2017). Past work by Grabowski et al. (2005) and Ziegler et al. (2018, 2021) in our system emphasized the role of habitat heterogeneity and connectivity at broader spatial scales. Of note, areas with highly interconnected marsh habitat tend to facilitate predation and trophic transfer (Ziegler et al., 2019), processes that enhance the flow of energy in food webs and are critical for parasite life cycle completion (Parker et al., 2015). For example, Stout et al. (2022) documented how trematode parasite communities changed at the landscape scale across multiple substrates (e.g., bare sand, seagrass), theorizing that differences in habitat have cascading effects on host populations. While habitat is undoubtedly important, future studies should quantify the extent to which habitat linkages or corridors affect parasite diversity using a key host like oyster toadfish.

Conclusions

We demonstrate that parasites can be a valuable tool for monitoring community diversity and trophic complexity in restored oyster reefs. Parasite diversity in xanthid crustaceans may provide better resolution of complexity at the site level, since these hosts are relatively immobile. Compared to preexisting natural reefs, parasite-driven differences in trophic complexity were most apparent beginning about 8 years after restoration. While most studies monitor for a maximum of 1–3 years (Baggett et al., 2015), our results suggest that complexity emerges over longer time intervals and may depend upon habitat context at the landscape scale. Given that toadfish and their parasites appear to act as sentinels of community

diversity, we suggest including these key host species (and their parasite infracommunities) in future reef monitoring studies. Collections should target larger toadfish in natural reefs to obtain a more complete picture of toadfish–parasite diversity in reference ecosystems.

ACKNOWLEDGMENTS

Funding for this work was provided to Christopher S. Moore by the Garden Club of America's 2019 Fellowship in Ecological Restoration; support and equipment from East Carolina University to April M. H. Blakeslee and Rachel K. Gittman, as well as an East Carolina University Natural Resources and Environment Research Cluster Seed grant to Rachel K. Gittman and April M. H. Blakeslee; and grants to Rachel K. Gittman from the North Carolina Department of Environmental Quality (No. NA15NOS4190091), National Oceanic and Atmospheric Administration (No. A19-0052-001), U.S. Fish and Wildlife Service (No. F19AP 00230), and the North Carolina Collaboratory (No. 20170 12593 221010 KOAST). We gratefully acknowledge the following individuals for their assistance: T. Boyd, C. Deaton, S. Donaher, M. Plafcan, S. Sutcliffe, S. Trackenberg, and E. Wellman. We thank Dr. Brandon Puckett for helping facilitate research within the Rachel Carson National Estuarine Research Reserve. Lastly, we appreciate the services of two anonymous reviewers whose suggestions improved the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Moore et al., 2023) are available from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (NOAA-NCEI) at https://www.ncei.noaa.gov/archive/accession/0276507.

ORCID

Christopher S. Moore https://orcid.org/0000-0003-0583-6176
Rachel K. Gittman https://orcid.org/0000-0001-8376-8960
April M. H. Blakeslee https://orcid.org/0000-0001-9667-2175

REFERENCES

Akaike, H. 1974. "A New Look at the Statistical Model Identification." *IEEE Transactions on Automatic Control* 19: 716–23.

Alboukadel, K. 2020. "rstatix: Pipe-Friendly Framework for Basic Statistical Tests." R Package Version 0.6.0. https://CRAN.R-project.org/package=rstatix.

Altieri, A., and J. von de Koppel. 2014. "Foundation Species in Marine Ecosystems." In *Marine Community Ecology and Conservation*, edited by M. Bertness, J. Bruno, and B. Silliman, 37–57. Sunderland, MA: Sinauer Associates, Inc.

Anderson, T., and M. Sukhdeo. 2011. "Host Centrality in Food Web Networks Determines Parasite Diversity." *PLoS One* 6: e26798.

Baggett, L. P., S. P. Powers, R. D. Brumbaugh, L. D. Coen, B. M. DeAngelis, J. K. Greene, B. T. Hancock, et al. 2015. "Guidelines for Evaluating Performance of Oyster Habitat Restoration." *Restoration Ecology* 23(6): 737–45.

Bayraktarov, E., S. Brisbane, V. Hagger, C. S. Smith, K. A. Wilson, C. E. Lovelock, C. Gillies, A. D. L. Steven, and M. I. Saunders. 2020. "Priorities and Motivations of Marine Coastal Restoration Research." *Frontiers in Marine Science* 7: 484.

Beck, M. W., R. D. Brumbaugh, L. Airoldi, A. Carranza, L. D. Coen, C. Crawford, O. Defeo, et al. 2011. "Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management." *Bioscience* 61(2): 107–16.

Benesh, D., J. Chubb, and G. Parker. 2014. "The Trophic Vacuum and the Evolution of Complex Life Cycles in Trophically Transmitted Helminths." *Proceedings of the Royal Society B* 281: 20141462.

Blakeslee, A. M., D. L. Pochtar, A. E. Fowler, C. S. Moore, T. S. Lee, R. B. Barnard, K. M. Swanson, et al. 2021. "Invasion of the Body Snatchers: The Role of Parasite Introduction in Host Distribution and Response to Salinity in Invaded Estuaries." *Proceedings of the Royal Society B* 288(1953): 20210703.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. "glmmTMB Balances Speed and Flexibility among Packages for Zero-Inflated Generalized Linear Mixed Modeling." *The R Journal* 9(2): 378–400.

Burnham, K. P., and D. R. Anderson. 2004. "Multimodel Inference: Understanding AIC and BIC in Model Selection." *Sociological Methods and Research* 33(2): 261–304.

Burt, J., A. Bartholomew, and P. Sale. 2011. "Benthic Development on Large-Scale Engineered Reefs: A Comparison of Communities among Breakwaters of Different Age and Natural Reefs." *Ecological Engineering* 37: 191–8.

Byers, J. E., A. M. Blakeslee, E. Linder, A. B. Cooper, and T. J. Maguire. 2008. "Controls of Spatial Variation in the Prevalence of Trematode Parasites Infecting a Marine Snail." *Ecology* 89(2): 439 451–1.

Caro, T. 2010. *Conservation by Proxy: Indicator, Umbrella, Keystone, Flagship, and Other Surrogate Species*. Washington, DC: Island Press.

Chase, J. M., B. J. McGill, D. J. McGlinn, F. May, S. A. Blowes, X. Xiao, T. M. Knight, O. Purschke, and N. J. Gotelli. 2018. "Embracing Scale-Dependence to Achieve a Deeper Understanding of Biodiversity and its Change across Communities." *Ecology Letters* 21(11): 1737–51.

Chen, H. W., W. C. Liu, A. J. Davis, F. Jordán, M. J. Hwang, and K. T. Shao. 2008. "Network Position of Hosts in Food Webs and their Parasite Diversity." *Oikos* 117(12): 1847–55.

Choisy, M., S. Brown, K. Lafferty, and F. Thomas. 2003. "Evolution of Trophic Transmission in Parasites: Why Add Intermediate Hosts?" *The American Naturalist* 162: 172–81.

Connor, E., and E. McCoy. 1979. "The Statistics and Biology of the Species–Area Relationship." *The American Naturalist* 113: 791–833.

Cushman, S. A., K. S. McKelvey, B. R. Noon, and K. McGarigal. 2009. "Use of Abundance of One Species as a Surrogate for Abundance of Another." *Conservation Biology* 24: 830–40.

Damgaard, C. 2019. "A Critique of the Space-for-Time Substitution Practice in Community Ecology." *Trends in Ecology and Evolution* 34: 416–21.

Esch, G., and J. Fernandez. 1993. *A Functional Biology of Parasitism*. New York: Chapman and Hall.

Fenton, A., D. G. Streicker, O. L. Petchey, and A. B. Pedersen. 2015. "Are All Hosts Created Equal? Partitioning Host Species Contributions to Parasite Persistence in Multihost Communities." *The American Naturalist* 186(5): 610–22.

Field, J., K. Clarke, and R. Warwick. 1982. "A Practical Strategy for Analyzing Multispecies Distribution Patterns." *Marine Ecology Progress Series* 8: 37–52.

Fodrie, F. J., A. B. Rodriguez, C. J. Baillie, M. C. Brodeur, S. E. Coleman, R. K. Gittman, D. A. Keller, et al. 2014. "Classic Paradigms in a Novel Environment: Inserting Food Web and Productivity Lessons from Rocky Shores and Saltmarshes into Biogenic Reef Restoration." *Journal of Applied Ecology* 51(5): 1314–25.

Froese, R., and D. Pauly. 2010. "Fishbase." http://www.fishbase.org.

Gain, I., R. Brewton, M. Robillard, K. Johnson, D. Smee, and G. Stunz. 2017. "Macrofauna Using Intertidal Oyster Reefs Varies in Relation to Position within the Estuarine Habitat Mosaic." *Marine Biology* 164: 8.

Grabowski, J., A. Hughes, and D. Kimbro. 2008. "Habitat Complexity Influences Cascading Effects of Multiple Predators." *Ecology* 89: 3413–20.

Grabowski, J., A. Hughes, D. Kimbro, and M. Dolan. 2005. "How Habitat Setting Influences Restored Oyster Reef Communities." *Ecology* 86: 1926–35.

Grabowski, J., and D. Kimbro. 2005. "Predator-Avoidance Behavior Extends Trophic Cascades to Refuge Habitats." *Ecology* 86: 1312–9.

Harding, J., D. Allen, E. Haffey, and K. Hoffman. 2019. "Site Fidelity of Oyster Reef Blennies and Gobies in Saltmarsh Tidal Creeks." *Estuaries and Coasts* 43: 409–23.

Hartig, F. 2022. "DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models." R Package Version 0.4.5. https://CRAN.R project.org/package=DHARMa.

Hechinger, R. F., and K. D. Lafferty. 2005. "Host Diversity Begets Parasite Diversity: Bird Final Hosts and Trematodes in Snail Intermediate Hosts." *Proceedings of the Royal Society B* 272: 1059–66.

Hechinger, R. F., K. D. Lafferty, T. C. Huspeni, A. J. Brooks, and A. M. Kuris. 2007. "Can Parasites be Indicators of Free-Living Diversity? Relationships between Species Richness and the Abundance of Larval Trematodes and of Local Benthos and Fishes." *Oecologia* 151(1): 82–92.

Hernández, A., R. D. Brumbaugh, P. Frederick, R. Grizzle, M. W. Luckenbach, C. H. Peterson, and C. Angelini. 2018. "Restoring the Eastern Oyster: How Much Progress Has Been Made in 53 Years?" *Frontiers in Ecology and the Environment* 16(8): 463–71.

Hill, C., M. Lymperaki, and B. Hoeksema. 2021. "A Centuries-Old Manmade Reef in the Caribbean Does Not Substitute for Natural Reefs in Terms of Species Assemblages and Interspecific Competition." *Marine Pollution Bulletin* 169: 112576.

Ho, J., T. Tumkaya, S. Aryal, H. Choi, and A. Claridge-Chang. 2019. "Moving beyond P Values: Data Analysis with Estimation Graphics." *Nature Methods* 16(7): 565–6.

Humphries, A. T., M. K. La Peyre, M. E. Kimball, and L. P. Rozas. 2011. "Testing the Effect of Habitat Structure and Complexity on Nekton Assemblages Using Experimental Oyster Reefs." *Journal of Experimental Marine Biology and Ecology* 409(1–2): 172–9.

Huspeni, T. C., and K. D. Lafferty. 2004. "Using Larval Trematodes that Parasitize Snails to Evaluate a Salt Marsh Restoration Project." *Ecological Applications* 14: 795–804.

Kamiya, T., K. O'Dwyer, S. Nakagawa, and R. Poulin. 2014. "Host Diversity Drives Parasite Diversity: Meta-Analytical Insights into Patterns and Causal Mechanisms." *Ecography* 37: 689–97.

Keller, D., R. Gittman, M. Brodeur, M. Kenworthy, J. Ridge, L. Yeager, A. Rodriguez, and J. Fodrie. 2019. "Saltmarsh Shoreline Geomorphology Influences the Success of Restored Oyster Reefs and Use by Associated Fauna." *Restoration Ecology* 27: 1429–41.

La Peyre, M., D. Marshall, L. Miller, and A. Humphries. 2019. "Oyster Reefs in Northern Gulf of Mexico Estuaries Harbor Diverse Fish and Decapod Crustacean Assemblages: Frontiers in Marine." *Science* 6: 1–13.

Lindenmayer, D., P. Barton, and J. Pierson. 2015. *Indicators and Surrogates of Biodiversity and Environmental Change*. Australia: CRC Press.

Linton, E. 1905. *Parasites of Fishes of Beaufort, North Carolina* 1904–21. Washington, DC: Bureau of Fishes. BBF.

Lüdecke, D., M. S. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. "Performance: An R Package for Assessment, Comparison and Testing of Statistical Models." *Journal of Open Source Software* 6(60): 3139. https://doi.org/10.21105/joss.03139.

Marcogoliese, D. 2002. "Food Webs and the Transmission of Parasites to Marine Fish." *Parasitology* 124: S83–99.

Mazerolle, M. J. 2020. "AICcmodavg: Model Selection and Multimodel Inference based on (Q)AIC(c)." R Package Version 2.3-1. https://cran.r-project.org/package=AICcmodavg.

McElreath, R. 2016. *Statistical Rethinking: A Bayesian Course with Examples in R and Stan*, 1st ed. Boca Raton, FL: Chapman and Hall/CRC.

Moore, C., C. Baillie, E. Edmonds, R. Gittman, and A. Blakeslee. 2023. "Species Abundance and Species Richness from 2019-07-02 to 2019-10-29 at Restored and Natural Oyster Reefs Located in Middle Marsh, Beaufort, N.C. (NCEI Accession 0276507)." NOAA National Centers for Environmental Information. Dataset. https://www.ncei.noaa.gov/archive/accession/0276507.

Moore, C. S., R. K. Gittman, B. J. Puckett, E. H. Wellman, and A. M. H. Blakeslee. 2020. "If You Build It, They Will Come: Restoration Positively Influences Free-Living and Parasite Diversity in a Restored Tidal Marsh." *Food Webs* 25: e00167.

Oksanen, J., G. L. Simpson, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, and R. B. O'Hara. 2020. "vegan: Community Ecology Package." R Package Version 2.5–7. https://CRAN.Rproject.org/package=vegan.

Parker, G., M. Ball, and J. Chubb. 2015. "Evolution of Complex Life Cycles in Trophically Transmitted Helminths. I. Host Incorporation and Trophic Ascent." *Journal of Evolutionary Biology* 28: 267–91.

Pasternak, A. F., F. A. Huntingford, and D. W. T. Crompton. 1995. "Changes in Metabolism and Behavior of the Freshwater Copepod *Cyclops strenuus abyssorum* Infected with *Diphyllobothrium* Spp." *Parasitology* 110(4): 395–9.

Perkol-Finkel, S., and Y. Benayahu. 2007. "Differential Recruitment of Benthic Communities on Neighboring Artificial and Natural Reefs." *Journal of Experimental Marine Biology and Ecology* 340(1): 25–39.

Poulin, R. 2007. *Evolutionary Ecology of Parasites*. Princeton, NJ: Princeton University Press.

Poulin, R., and T. Leung. 2011. "Body Size, Trophic Level, and the Use of Fish as Transmission Routes by Parasites." *Oecologia* 166: 731–8.

Rezek, R., B. Furman, R. Jung, M. Hall, and S. Bell. 2019. "Long-Term Performance of Seagrass Restoration Projects in Florida, USA." *Scientific Reports* 9: 15514.

Ridge, J., A. Rodriguez, and F. Fodrie. 2017. "Saltmarsh and Fringing Oyster Reef Transgression in a Shallow Temperate Estuary." *Estuaries and Coasts* 40: 1013–27.

Rossiter, W., and M. Sukhdeo. 2014. "Habitat-Based Constraints on Food Web Structure and Parasite Lifecycles." *Oecologia* 174: 1311–21.

Schwartz, F., and B. Dutcher. 1963. "Age, Growth and Food of the Oyster Toadfish near Solomon's, Maryland." *Transactions of the American Fisheries Society* 92: 170–3.

Smith, R., B. Lusk, and M. Castorani. 2022. "Restored Oyster Reefs Match Multiple Functions of Natural Reefs within a Decade." *Conservation Letters* 15: e12883.

Stone, B., and J. Brown. 2018. *Sampling Nektonic Organisms around Restored Oyster Reefs in the South Atlantic: A Practitioner's Guide to Regional Sampling*. Charleston, SC: The Nature Conservancy, SC-DNR.

Stout, L., A. Garenne, and X. de Montaudouin. 2022. "Marine Trematode Parasites as Indicators of Environmental Changes." *Ecological Indicators* 141: 109089.

Thompson, R. M., K. N. Mouritsen, and R. Poulin. 2005. "Importance of Parasites and their Lifecycle Characteristics in Determining the Structure of a Large Marine Food Web." *Journal of Animal Ecology* 74(1): 77–85.

Toscano, B., B. Newsome, and B. Griffen. 2014. "Parasite Modification of Predator Functional Response." *Oecologia* 175: 345–52.

U.N. 2021. "Decade on Ecosystem Restoration: 2021–2030." https://www.decadeonrestoration.org/.

Wiens, J. A., G. D. Hayward, R. S. Holthausen, and M. J. Wisdom. 2008. "Using Surrogate Species and Groups for Conservation Planning and Management." *Bioscience* 58: 241–52.

Wilson, C., J. Dean, and R. Radke. 1982. "Age, Growth Rate and Feeding Habits of the Oyster Toadfish, *Opsanus tau* (Linnaeus) in South Carolina." *Journal of Experimental Marine Biology and Ecology* 62: 251–9.

Wogan, G., and I. Wang. 2017. "The Value of Space-for-Time Substitution for Studying Fine-Scale Microevolutionary Processes." *Ecography* 41: 1456–68.

Wood, C., and P. Johnson. 2016. "How Does Space Influence the Relationship between Host and Parasite Diversity?" *Journal of Parasitology* 102: 485–94.

Yamaguti, S. 1971. *Synopsis of Digenetic Trematodes of Vertebrates*. Tokyo: Keigaku Publishing.

Ziegler, S., K. Able, and J. Fodrie. 2019. "Dietary Shifts across Biogeographic Scales Alter Spatial Subsidy Dynamics." *Ecosphere* 10: e02980.

Ziegler, S., L. Clance, A. McMains, M. Miller, and J. Fodrie. 2021. "Influence of Marsh Island Size on Nekton Communities: Intermediate Optima Rather than Several Large or Single Small (SLOSS)." *Marine Ecology Progress Series* 672: 45–56.

Ziegler, S. L., J. H. Grabowski, C. J. Baillie, and F. J. Fodrie. 2018. "Effects of Landscape Setting on Oyster Reef Structure and Function Largely Persist More than a Decade Post Restoration." *Restoration Ecology* 26(5): 933–42.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Moore, Christopher S., Christopher J. Baillie, Emily A. Edmonds, Rachel K. Gittman, and April M. H. Blakeslee. 2023. "Parasites Indicate Trophic Complexity and Faunal Succession in Restored Oyster Reefs over a 22-Year Period." *Ecological Applications* 33(4): e2825. https://doi.org/10.1002/eap.2825