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Host genetic identity determines parasite community structure across time and space in oyster restoration

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Intraspecific variation in host susceptibility to individual parasite species is common, yet how these effects scale to mediate the structure of diverse parasite communities in nature is less well understood. To address this knowledge gap, we tested how host genetic identity affects parasite communities on restored reefs seeded with juvenile oysters from different sources—a regional commercial hatchery or one of two wild progenitor lines. We assessed prevalence and intensity of three micro- and two macroparasite species for 4 years following restoration. Despite the spatial proximity of restored reefs, oyster source identity strongly predicted parasite community prevalence across all years, with sources varying in their relative susceptibility to different parasites. Oyster seed source also predicted reef-level parasite intensities across space and through time. Our results highlight that host intraspecific variation can shape parasite community structure in natural systems, and reinforce the importance of considering source identity and diversity in restoration design.

1. Introduction

Parasites are ubiquitous and diverse in ecological communities [1,2], with the capacity to alter population, community and ecosystem processes across multiple scales [3–5]. Understanding the factors that shape host–parasite interactions is thus critical, particularly in the face of increasing disease outbreaks [6,7]. Host intraspecific variation is one such factor, influencing host–parasite dynamics both within and across host populations [8,9]. For example, intraspecific diversity within host populations is often negatively associated with infection prevalence due to a variety of non-exclusive mechanisms, including greater susceptibility of genetically homogeneous monocultures [10], a 'dilution effect' of genetically diverse populations decreasing encounter rates (reviewed in [11]) and limiting disease spread [12], as well as higher parasite transmission among genetically related hosts [13]. Across populations, variation in host susceptibility is common [14] due to genetically based factors such as population history of infection [15], ecotypic variation [16] and resistance trade-offs [17], environmentally driven factors such as resource quantity/quality, nutrient availability and disease pressure [18,19], or a combination of both [20]. Disentangling genetic and environmental drivers of variation in host–parasite interactions across populations can be challenging in non-model systems given the necessary scale of manipulations, yet it is key to effective management and conservation initiatives.

Variation in host susceptibility across populations can arise from differences in traits that prevent infection or limit parasite replication (i.e. resistance [21–23]) and/or in traits that mitigate disease severity and effects on fitness (i.e. tolerance [24–26]). These differences in host resistance or tolerance can have distinct consequences for host–parasite interactions [27], as well as critical implications for management and conservation [28], especially when host populations provide important ecosystem functions and services [29]. Measuring multiple infection metrics, including both prevalence (proportion of infected hosts) and intensity (parasite concentration within infected hosts), can provide insight into the mechanisms underlying host–parasite dynamics (e.g. [30]). For example, with low mortality, high population prevalence and intensity would suggest relatively low resistance and relatively high tolerance, whereas low population prevalence and intensity would suggest greater resistance. By contrast, high mortality probably indicates both low resistance and low tolerance of the host population. In addition, co-infection of hosts by multiple micro- and macroparasites is common [31,32], and mechanisms of host resistance and tolerance can be parasite species- or strain-specific [33]. Thus, examining variation in prevalence and intensity across multiple parasites can provide critical information on and necessary context for the overall susceptibility of host populations to parasites.

Habitat restoration provides an opportunity to examine factors that can affect host–parasite dynamics in a large-scale, real-world context, as well as test their consequences for short- and long-term restoration success. In particular, parasites that infect habitat-providing species, which are often the focus of restoration, may have a disproportionate effect on restoration success and in turn ecosystem function [34,35]. Because host intraspecific diversity is a key potential driver of host–parasite interactions, manipulating genetic variation of these target species could help inform metrics of restoration success and improve predictions of long-term community stability [36–39]. Consideration of genetic variation, including the identity or number of different sources (e.g. populations, cultivars), is increasingly recognized as important to restoration practice [40–44]. However, relatively few published restoration efforts have manipulated genetic variation at the design/implementation stage of restoration [45], and even fewer consider the effects of host genetic variation on parasite dynamics (but see [19] for examples from agricultural systems), despite the recognized threat that diseases pose to habitat restoration success [46,47].

We examined how host intraspecific variation affects parasite community structure on experimentally restored oyster (*Crassostrea virginica*) reefs. *Crassostrea virginica* is a target species for habitat restoration efforts throughout its range along the Atlantic and Gulf coasts of the United States because it provides numerous ecologically important and socioeconomically valuable ecosystem functions and services [48,49]. Oysters are susceptible to a variety of micro- and macroparasites, and they are commonly co-infected by multiple species in temperate and tropical systems [50–55], which can influence restoration success in the short- and long-term. In regions where oyster recruitment is limited and/or settlement is unpredictable, restored reefs are often 'seeded' with hatchery-produced juvenile oysters [56,57], providing an opportunity to manipulate host intraspecific identity and examine its effects on parasite community assembly and structure. Here, we assessed prevalence, intensity, and community structure of five micro- and macroparasites annually for four years post-restoration on replicate reefs seeded with spat on shell from one of three oyster sources. We hypothesized that the source identity of oysters used to seed each reef would determine prevalence and intensity of this multi-parasite community, with implications for restoration success and practice.

2. Methods

(a) Study system

Given significant declines in oyster abundances and reef habitat extent and quality worldwide [58–61], oyster reef restoration has increased globally [62,63]. Methods commonly include (i) substrate addition/supplementation to facilitate natural oyster settlement and reef accretion, and/or (ii) juvenile oyster (spat) seeding, particularly in regions with limited larval supply. The latter practice provides a valuable opportunity to explore the effects of oyster genetic identity and diversity in the form of different seed sources with unique population histories (e.g. commercial hatchery lines versus wild progenitor cohorts; [64,65]). While small-scale manipulations indicate that oyster seed source variation underlies differences in population characteristics and performance [66–68], whether these effects scale up to the reef level and impact community dynamics requires examination.

In Rhode Island (RI; the location of this study), seeding of private oyster leases was prevalent from 1910 to the mid-1930s using oysters sourced from Long Island Sound to Chesapeake Bay due to limited and variable recruitment in the region, as well as high demand for oysters [69]. In the late 1930s, RI oyster populations plummeted as a result of increased sedimentation, decades of overharvest, and the Great New England Hurricane of 1938, and they now remain at 1% of their historical abundances [60,69]. The few populations that have persisted for the past 75+ years represent the 'wild' extant oysters in the region. Although oyster reef restoration efforts have been ongoing over the past couple of decades in RI, low rates of natural recruitment have remained a barrier to restoration success (e.g. [70,71]). Identifying viable source populations could be critical to overcoming this bottleneck and successfully restoring RI oyster populations.

(b) Oyster reef restoration experiment

In May 2017, we created nine oyster reefs in Quonochontaug Pond, Rhode Island, with three separate reefs each located within three distinct regions (west, northeast, east; hereafter referred to as blocks; figure 1). Each approximately 22 m^2 reef (0.5–0.8 m height) was constructed from a base layer of steam-shucked clam shell topped with clean, recycled oyster shell, and then seeded with remote-set spat on shell (see [72] for a detailed description of reef construction). Each block also included an adjacent control plot with soft sediment (mud and/or sand) that was unmodified (i.e. no shell or spat added), and treatments and controls were randomly assigned within blocks. A goal of this study was to compare the performance of different oyster sources and the consequent structure of associated parasite communities, so one replicate reef per oyster source was constructed in each of the three regions of Quonochontaug Pond (figure 1). The oyster seed sources included one line from a regional commercial hatchery, as well as two wild progenitor lines spawned from broodstock collected from nearby existing wild populations in Green Hill Pond, RI and Narrow River, RI. All oyster lines were spawned at local hatcheries, set on oyster shell at Roger Williams University Shellfish Hatchery in June

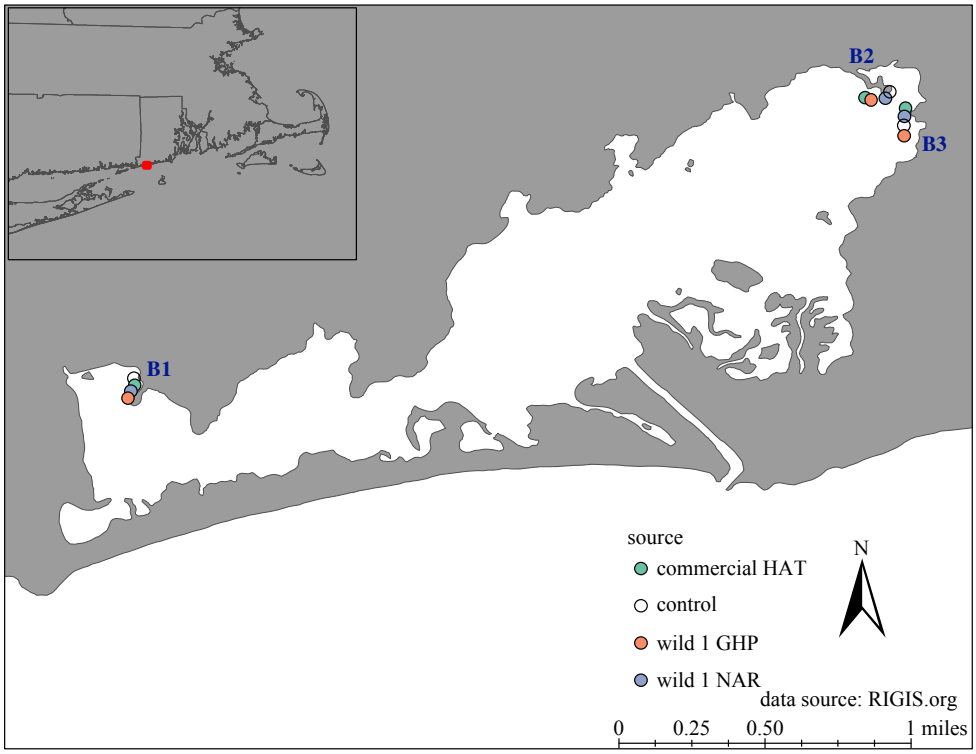

Figure 1. Map of restored oyster reefs (represented by filled circles) in Quonochontaug Pond, RI, USA seeded with spat on shell from different sources (commercial hatchery (HAT), green; Green Hill Pond (GHP), orange; Narrow River (NAR), blue; control (adjacent unmodified plot with soft sediment; no spat or shell added), open circles) across blocks (B1, B2, B3) in spring 2017.

2016, and then stored in cages on an oyster lease in Quonochontaug Pond until reef construction in May 2017.

To confirm genetic differentiation of the oyster lines, we sampled 48 oysters per seed source ($n = 144$ total) prior to deployment and genotyped them using 20 highly variable microsatellite loci (see electronic supplementary material, appendix S1 for detailed methods and analyses). We found significant genetic differentiation among seed sources (electronic supplementary material, appendix S1 and table S2), with spat produced from wild progenitors (Green Hill Pond and Narrow River) being more similar to each other than to spat from the commercial hatchery (electronic supplementary material, appendix S1 and figure S1). Genetic diversity also varied among seed sources, with spat from wild progenitors having higher genetic diversity than spat from the commercial hatchery (electronic supplementary material, appendix S1 and table S1).

(c) Reef monitoring and oyster collection

In the autumn of each year for 4 years post-restoration (2017–2020), we monitored oyster density and size distribution by non-destructively sampling six haphazard 0.25 m^2 quadrats per reef and recording the number of live and dead oysters, as well as shell height of a subsample of up to 50 live and 30 dead oysters (following methods of [73] and guidance of [74]). Coincident with each autumn monitoring event, we harvested approximately 35 haphazardly selected live oysters from each reef for analysis of parasite communities; samples were transported to the Northeastern University Marine Science Center on ice and then stored at −80°C prior to processing.

(d) Parasite prevalence and intensity

Crassostrea virginica is commonly infected by a variety of micro- and macroparasites simultaneously. We assessed the prevalence (proportion of sampled oysters infected per oyster reef) and intensity (parasite concentration per infected host) of five common parasite species (microparasites: *Perkinsus marinus*, *Haplosporidium nelsoni* and *Haplosporidium costale*; macroparasites: *Cliona* spp. and *Polydora* spp.). The protozoan parasite *P. marinus* causes dermo disease, which has been associated with decreased oyster growth, reproduction, and condition [75], as well as mass oyster mortality events along the Atlantic and Gulf coasts of the USA [76,77]. The microparasite *H. nelsoni* is the causative agent of MSX disease; while MSX has devastated some *C. virginica* populations along the Atlantic Coast, its effects have been less extreme in some regions, due in part to development of disease resistance [78–80]. The protistan parasite *H. costale*, which is morphologically similar to *H. nelsoni*, causes SSO disease in oyster populations along the Atlantic Coast [81], though it is most often associated with mass mortalities under high salinity (greater than 25 ppt) conditions in late spring and early summer [82]. Boring sponges (*Cliona* spp.) are macroparasites common along the Atlantic and Gulf coasts that impact oyster growth and condition, compromise shell integrity and decrease marketability, but are rarely associated with mass mortality events [83–85]. Shell-boring polychaetes like mud blister worms (*Polydora* spp.) are prevalent along the Atlantic and Gulf coasts; similar to boring sponges, they often result in decreased size and condition of oyster hosts, but do not consistently cause mass mortalities [50,86,87].

To assess macroparasite prevalence and intensity, we photographed the inside and outside of the top and/or bottom valves of all oysters with holes characteristic of boring sponges and/or blisters indicating the presence of mud blister worms, and then quantified the proportion of affected shell area using ImageJ [53,88] (see electronic supplementary material, appendix S2 for detailed methods). To assess microparasite prevalence and intensity, DNA was extracted from up to 32 oysters per reef using the Omega Bio-Tek E-Z 96 Tissue DNA Kit and then amplified using both a polymerase chain reaction (PCR) assay modified from Stokes & Burreson's SSO protocol [81], and a quantitative polymerase chain reaction (qPCR) assay modified from De Faveri *et al.*'s dermo protocol [89] and Wilbur *et al.*'s MSX protocol

[80] (see electronic supplementary material, appendix S2 for detailed methods). We have previously validated the *P. marinus* qPCR assay with standard Ray's fluid thioglycolate medium (RFTM) histological assays to confirm presence/absence of hypnospores and quantify infection intensity [53]. We did not conduct equivalent histological assays for *H. nelsoni* or *H. costale* due to the difficulty of distinguishing among them, as well as the limited ability to detect early nucleated/spore forms at low to moderate levels of infection [90–92].

(e) Statistical analysis

First, we used a permutational multivariate analysis of variance (PERMANOVA) to test for differences in parasite community composition (i.e. reef-level prevalence and intensity of micro- and macroparasites) across reefs seeded with different oyster sources, among regions (i.e. blocks) within Quonochontaug Pond, and through time. Specifically, we focused on the main effects of each factor along with year × seed source and block × seed source interactions to assess the independent and interactive effects of seed source, time and reef location on parasite communities, and to evaluate the temporal and spatial consistency of seed source effects (see results of PERMANOVA examining the effects of year × block in electronic supplementary material, appendix S3).

Second, we used non-metric multidimensional scaling (nMDS) to visualize (i) significant effects of seed source, block and year on oyster parasite community prevalence and intensity based on PERMANOVA results, and (ii) temporal dynamics of parasite communities in ordination space across the years following reef restoration (2017–2020 for prevalence and 2018–2020 for intensity). For prevalence, we included all micro- and macroparasite species (*H. costale*, *H. nelsoni*, *P. marinus*, *Cliona* spp. and *Polydora* spp.) across years (2017–2020). For intensity, we excluded the first year following restoration (2017) because samples from four of the nine reefs had zero prevalence of at least one parasite species, resulting in no intensity data for those reef × parasite combinations and a loss of statistical power. In subsequent years (2018–2020), we focused only on *H. costale*, *P. marinus*, *Cliona* spp. and *Polydora* spp. for intensity analyses given zero to very low prevalence of *H. nelsoni* throughout the study. We used percent cover (i.e. [(total infected area/total shell area) × 100]) as our intensity metric for boring sponge and mud blister worm infections. To account for differences in mean and range among intensities of parasite species, we fourth-root transformed dermo intensity data (concentration of *P. marinus* per mg oyster tissue) prior to analysis [93].

Given that PERMANOVA identified significant effects of seed source and time, but not of block, on parasite community prevalence (see Results and electronic supplementary material, appendix S3 and figure S2), we then focused on the independent effects of (i) seed source and (ii) two host characteristics (oyster density and oyster size) on parasite prevalence in each year of the study to assess which of these best explained differences in parasite prevalence among restored reefs. Because changes in density and size following reef establishment (electronic supplementary material, appendix S4 and figure S3) reflected differences in oyster mortality and growth among reefs [94], we examined whether these host characteristics contributed to the significant effects of time in our analysis. Parasite community data included five micro- and macroparasites in 2018 and 2019, but only four micro- and macroparasites in 2017 and 2020 given zero prevalence of *H. nelsoni* (MSX) in the first and last years of the study. For both the comparison across years and the analysis of individual years, we used metaMDS and adonis in the vegan package [95] in R version 3.5.3 to produce nMDS plots and conduct PERMANOVA, respectively.

Given that it is difficult to differentiate between infected oyster tissue versus environmental parasite DNA for *H. nelsoni* or *H. costale*, we conducted a second set of statistical analyses excluding these parasites to examine the consistency of our results (see electronic supplementary material, appendix S6 for details). In short, the main predictor(s) of parasite community prevalence and intensity were consistent with and without *H. nelsoni* and *H. costale*. Thus, we present the results including all micro- and macroparasite species in the main text for completeness, and we report the results using only three parasite species in electronic supplementary material, appendix S6 for comparison.

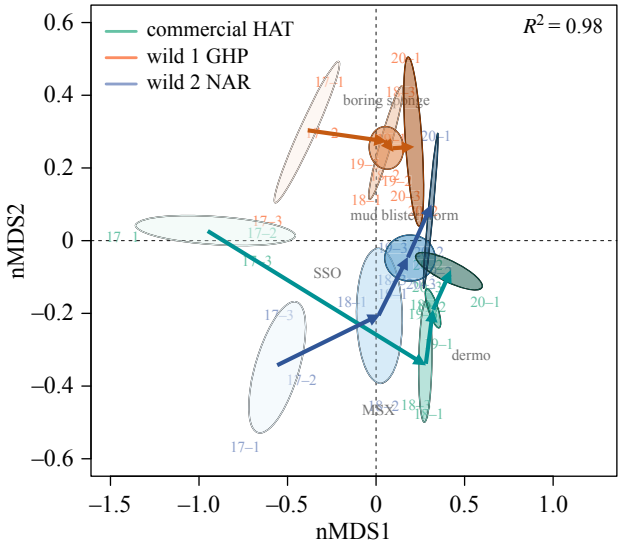

Figure 2. Non-metric multidimensional scaling (nMDS) plot depicting prevalence of microparasites *P. marinus* (dermo), *H. costale* (SSO) and *H. nelsoni* (MSX), and macroparasites *Cliona* spp. (boring sponge) and *Polydora* spp. (mud blister worm), through time (lightest to darkest shades corresponding to 2017, 2018, 2019 and 2020, respectively) on oyster reefs restored with different seed sources (commercial hatchery (HAT), green; Green Hill Pond (GHP), orange; Narrow River (NAR), blue); ellipses include 95% confidence intervals depicting year × seed source effects. Individual datapoints represent year–block combinations (e.g. orange 17–1 is block 1 GHP-seeded reef in 2017) and arrows depict trends in mean parasite community prevalence through time.

3. Results

(a) Parasite community prevalence

The prevalence of micro- and macroparasite communities (including all five parasites) on restored oyster reefs over the time course of the experiment depended interactively on seed source and year (PERMANOVA: year × seed source, $p = 0.008$; figure 2). When we examined years separately, there was a significant effect of oyster seed source on parasite community prevalence in each year (PERMANOVA: seed source: 2017, $p = 0.016$; 2018, $p = 0.009$; 2019, $p = 0.001$; 2020, $p = 0.028$; figure 3). In the first two years after the restoration, neither oyster density (PERMANOVA: 2017, $p = 0.772$; 2018, $p = 0.276$) nor oyster size (PERMANOVA: 2017, $p = 0.280$; 2018, $p = 0.644$) were correlated with parasite community structure. However, oyster density was strongly correlated with parasite prevalence in 2019 and 2020 (PERMANOVA: 2019, $p = 0.004$; 2020, $p = 0.027$): reefs with higher oyster densities had greater boring sponge prevalence, intermediate mud blister and SSO prevalence, and lower dermo and MSX prevalence (figure 3*c*,

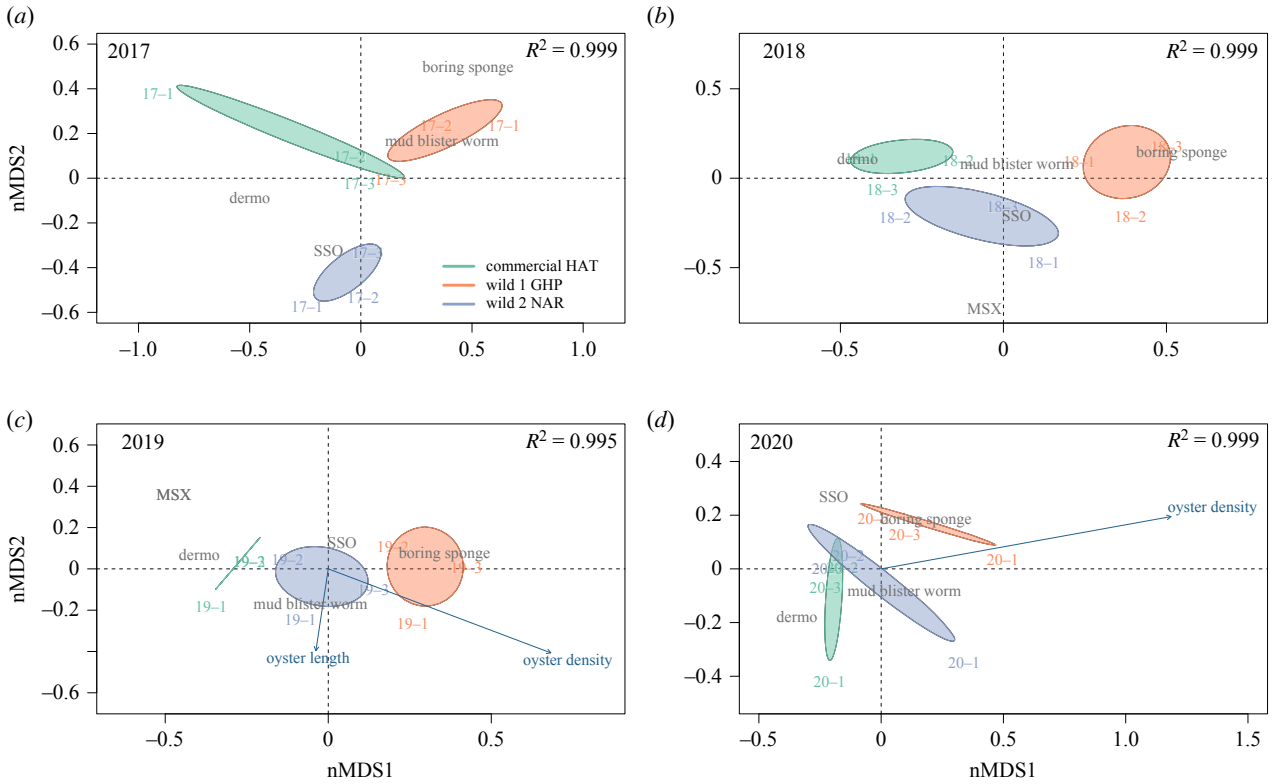

Figure 3. Non-metric multidimensional scaling (nMDS) plots depicting prevalence of microparasites *P. marinus* (dermo), *H. costale* (SSO) and *H. nelsoni* (MSX), and macroparasites *Cliona* spp. (boring sponge) and *Polydora* spp. (mud blister worm), on oyster reefs restored with different seed sources (commercial hatchery (HAT), green; Green Hill Pond (GHP), orange; Narrow River (NAR), blue) in (*a*) 2017, (*b*) 2018, (*c*) 2019, and (*d*) 2020; ellipses include 95% confidence intervals depicting seed source effects and vectors represent explanatory variables ($p < 0.05$; oyster density and oyster length) correlated to the axes. Individual datapoints denote seed source–year–block combinations (e.g. in (*a*), blue 17–1 represents block 1 NAR-seeded reef in 2017). In 2019 (*c*), HAT-seeded reefs in blocks 2 and 3 overlap/occupy approximately the same position in multivariate space.

d). In 2019, oyster size was also a strong correlate of parasite community structure (PERMANOVA: $p = 0.012$): reefs with larger oysters had higher mud blister worm prevalence, whereas reefs with smaller oysters had higher SSO, dermo and boring sponge prevalence (figure 3*c*).

The largest shifts in parasite communities occurred from 2017 to 2018, with changes in parasite community prevalence being more pronounced for reefs seeded with spat from a commercial hatchery than for reefs seeded with spat from wild progenitors (figure 2). From 2018 to 2019, parasite community structure among reefs with the same seed source shifted relatively little (figure 2). By 2020, parasite communities had started to converge across reefs (figure 2)—probably as a result of overall greater parasite prevalence (figure 4)—though as noted above, there were still significant differences among reefs seeded with oyster spat from different sources.

(b) Parasite community intensity

From 2018 to 2020, average micro- and macroparasite intensities on restored reefs also differed consistently by seed source, with no independent or interactive effects of year (PERMANOVA: seed source, $p = 0.002$; year, $p = 0.186$; year × seed source, $p = 0.747$; figure 5). Reefs seeded with spat from a commercial hatchery had generally higher *P. marinus* concentrations (dermo) than those seeded with spat from wild progenitors. By contrast, reefs seeded with spat from wild Narrow River and Green Hill Pond broodstock had generally higher *H. costale* concentrations (SSO disease) or boring sponge percent cover, respectively (figure 5). Mud blister worm intensities were more variable among sources and across years.

(c) Spatial (block) effects

Across all years, the effects of seed source on parasite community prevalence were consistent across the three spatial blocks (PERMANOVA, seed source: $p = 0.002$, block: $p = 0.577$, block × seed source: $p = 0.969$; electronic supplementary material, appendix S4 and figure S4). Similarly, the effects of seed source on parasite community intensity from 2018 to 2020 were consistent across space, with significant effects of seed source, only marginal effects of block, and no interactive effects of block × seed source on reef-level intensities (PERMANOVA, seed source: $p = 0.002$, block: $p = 0.071$, block × seed source: $p = 0.456$).

4. Discussion

In our experimental restoration, oyster genetic identity had strong and persistent effects on parasite community prevalence and intensity (figures 2 and 5). Notably, these effects of oyster seed source were not transient, but detectable within the first six months of the study and persistent for four years following reef restoration, with host characteristics—specifically density and size—also emerging as significant correlates of prevalence in later years of the study (figure 3). Further, the effects of oyster seed source on parasite community prevalence and intensity did not vary spatially across the three distinct regions of the Quonochontaug Pond

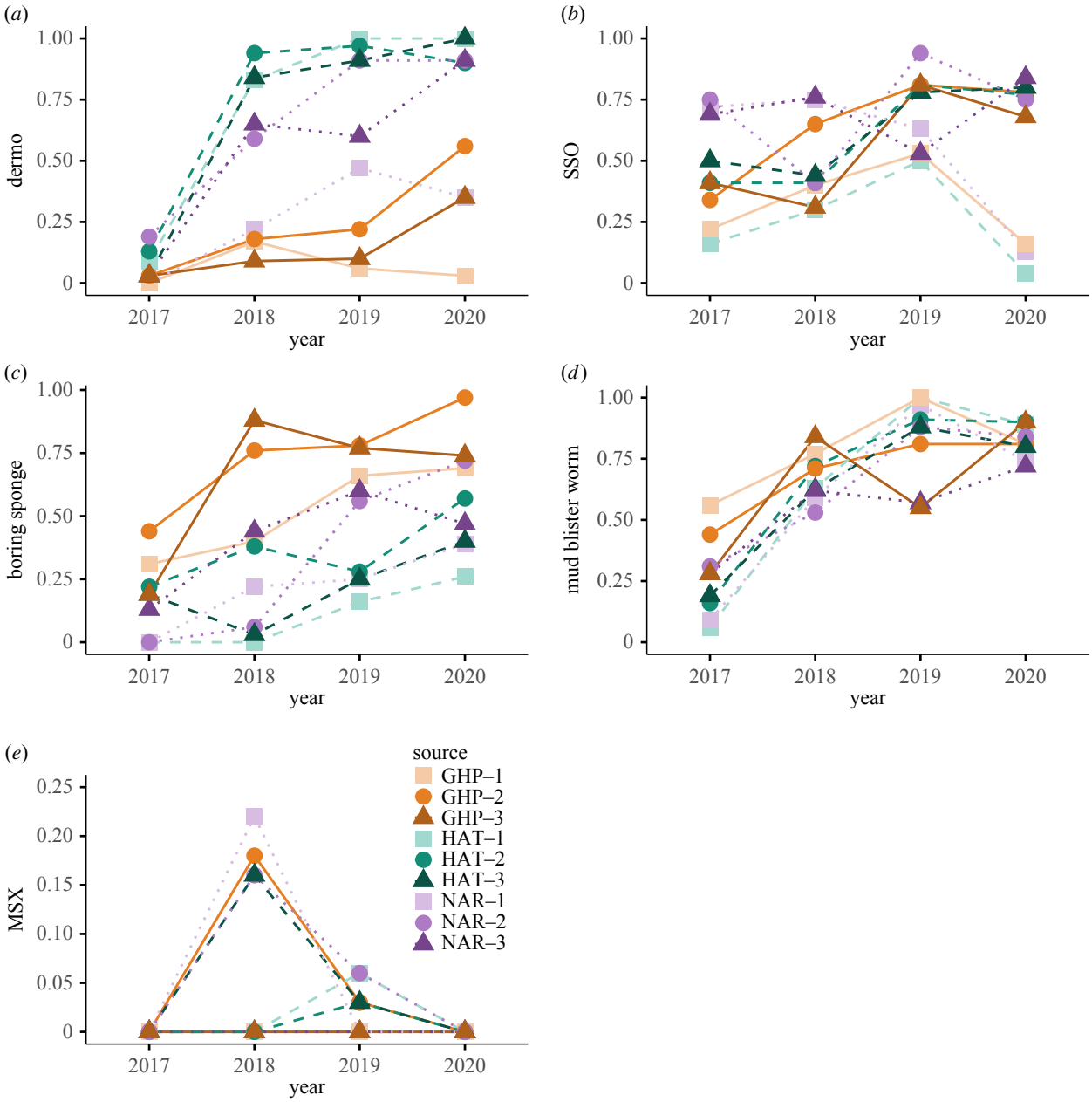

Figure 4. Prevalence of microparasites (*a*) *P. marinus* (dermo), (*b*) *H. costale* (SSO) and (*e*) *H. nelsoni* (MSX), and macroparasites (*c*) *Cliona* spp. and (*d*) *Polydora* spp., on oyster reefs restored with different seed sources (commercial hatchery (HAT), green; Green Hill Pond (GHP), orange; Narrow River (NAR), purple) across blocks (1, 2 and 3 represented by light to dark shades and squares, circles and triangles, respectively) in Quonochontaug Pond, Rhode Island for the years following the restoration (2017–2020). Note the different *y*-axis in panel (*e*) (0–0.25).

restoration. Finally, the results were consistent when looking at a three-species subset of the larger five-species community (see electronic supplementary material, appendix S6 for details). These findings complement studies on a variety of plant and animal species in aquatic and terrestrial systems that have demonstrated effects of host genetic variation on individual parasite dynamics (e.g. [8,9,96]), as well as those that have detected associations between host genetic variation and parasite species diversity and community assembly in natural settings [97,98] where co-infection is ubiquitous [31]. Importantly, our study also highlights that host genetic identity drives differences in susceptibility to a diverse suite of micro- and macroparasites—a key finding that informs restoration and management practices and would have been missed by focusing on a single parasite or genetic line.

Host genetic variation may be particularly important to consider in predictions of parasite community dynamics for systems dominated by one or a few species, either naturally (e.g. coastal and estuarine systems with foundation species, such as seagrass meadows, salt marshes and oyster reefs) or as a result of human activities (e.g. agricultural fields, aquaculture farms, early stages of restoration projects with focal species) [8,9,19,28]. Differences in host genetic identity, like those observed among oyster seed sources in this study (electronic supplementary material, appendix S1), can result in intraspecific variation in resistance and/or tolerance traits [27], as well as in behavioural defense mechanisms against parasites [99]. For example, selectively bred oyster families modified their feeding behaviour to differing degrees in the presence of *P. marinus*, and these behavioural changes correlated with varying levels of susceptibility (e.g. the most susceptible family reduced its feeding rate the least and thus had greater risk of encountering parasites [100]). While this defense strategy may be effective against microparasites,

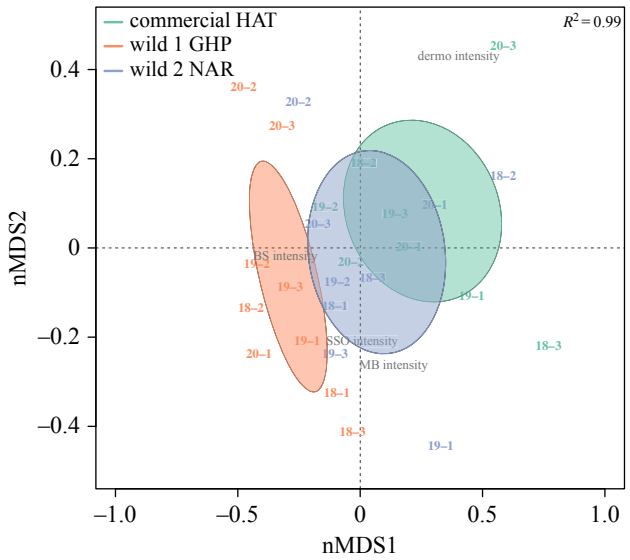

Figure 5. Non-metric multidimensional scaling (nMDS) plot depicting reef-level intensities of microparasites *P. marinus* (dermo) and *H. costale* (SSO), and macroparasites *Cliona* spp. (boring sponge) and *Polydora* spp. (mud blister worm), from 2018 to 2020 on oyster reefs restored with different seed sources (commercial hatchery (HAT), green; Green Hill Pond (GHP), orange; Narrow River (NAR), blue); ellipses include 95% confidence intervals depicting seed source effects. Individual datapoints represent year-block combinations (e.g. green 20-3 represents block 3 hatchery-seeded reef in 2020).

changes in feeding behaviour will not reduce exposure to macroparasites like boring sponges and mud blister worms, and may limit oyster capacity for tolerance mechanisms such as shell repair, which require increased energy expenditure [84,85,101]. For example, co-infection by the microparasite *H. nelsoni* and the macroparasite *Polydora* spp. can amplify the negative effects of each individual parasite, with parasite intensities of co-infected oysters associated with decreases in oyster condition index [50]. These differences in optimal host behavioural strategies for avoidance of micro- versus macroparasites further illustrate why it is unlikely that a single oyster source will be universally resistant to or tolerant of all parasites [102]. In addition, host genetic identity and parasite co-infection status can impact transmission rates, with certain hosts having greater among-host (alloinfection) transmission [103], further reinforcing initial differences in parasite prevalence and potentially contributing to the persistent differences among reefs with different seed sources in our experiment (figure 3).

Host intraspecific variation in parasite susceptibility can also reflect differences in past selection regimes, particularly when hosts are sourced from populations with distinct parasite communities that may have generated divergent selective pressures favouring different traits. In our study, the wild progenitors came from populations with distinct macroparasite communities: the Green Hill Pond population had greater boring sponge prevalence, whereas the Narrow River population had greater mud blister worm prevalence (electronic supplementary material, figure S6 and appendix S5 for details). While parasite prevalence and susceptibility are often higher for relatively naive than previously exposed host populations [104,105], we found the opposite pattern for macroparasites on restored reefs, with Green Hill Pond sourced reefs having high boring sponge prevalence and Narrow River sourced reefs having high mud blister worm prevalence (figure 4). This pattern may reflect evolved differences in host tolerance that alleviate the fitness consequences of infection, as opposed to evolved differences in host resistance that prevent infection [106,107]. Higher boring sponge intensities on Green Hill Pond sourced reefs, particularly in the last 2 years of the study, corroborate this idea (figure 5).

Host genetic variation (including genetic identity) may impact the success of microparasites more than macroparasites [8], with host density instead being a key predictor of macroparasite dynamics [8,108]. In the latter years of our study, micro- and macroparasites were largely separated from each other along the first axis of the nMDS plots (figure 3): host density was positively correlated with macroparasite prevalence and negatively correlated with microparasite prevalence. This result may be due to density-dependent transmission (a mechanism less explored for macroparasites than microparasites; but see [109]), greater mortality associated with microparasites, greater morbidity associated with macroparasites or a combination of all of the above [110]. In addition, evolution of host resistance may be more common in response to microparasites than macroparasites given greater likelihood of the former to induce a sustained immune response in the host [107,111], increasing the probability of a relationship between host genetics and microparasite dynamics. Resistance to MSX disease has developed among wild oyster populations along the Atlantic Coast of the USA, including Delaware Bay [78] and Chesapeake Bay [79], contributing to a decline in MSX prevalence. Evolution of disease resistance may have also contributed to low prevalence and intensity of *H. nelsoni* on reefs seeded with spat from wild progenitors in our study, though MSX dynamics at sites in the region have historically exhibited substantial annual variation [67], probably limiting selection for resistance. How past selection regimes impact resistance versus tolerance strategies for different genetic lines, and whether host responses differ for micro- versus macroparasite species, are fruitful areas for further study in this system.

Selective breeding practices are being employed across a range of settings (e.g. agriculture, aquaculture) to maintain or enhance productivity of key host species at risk of encountering multiple pathogens, parasites and diseases [112,113]. For example, commercial oyster lines have been selectively bred for a wide range of traits, including disease resistance [67,114–116], typically with an emphasis on host resistance to a single parasite (but see oyster DEBY line [117]) or optimal performance under specific environmental conditions. This approach can be effective at local sites with high endemicity of a specific parasite [118], but it can also limit success of a single line when deployed across a range of abiotic and biotic conditions with variable selection pressures [67]. Further, the selective breeding process may contribute to lower genetic diversity in commercial/hatchery lines relative to wild populations (electronic supplementary material, appendix S1 and table S1) [64]. To our knowledge, the hatchery line used in this study was not specifically selected for disease resistance. Reefs seeded with this local, commercial source had high prevalence of the microparasite *P. marinus* (dermo) and generally low prevalence of the macroparasite species, as well as overall lower oyster densities than reefs seeded with wild progenitor sources. Additional work is needed comparing multiple commercial oyster lines with those produced from wild progenitors to determine if the differences

we observed in micro- and macroparasite prevalence among source types are consistent.

Our findings demonstrate strong and persistent effects of host genetic identity on parasite community structure and dynamics on restored oyster reefs. The key takeaways for restoration practice are that source population matters, and there is probably no single source population that is most resistant to or tolerant of the diverse suite of parasite species commonly encountered in natural systems. Thus, using multiple sources when feasible may be an effective 'hedging your bets' strategy to ensure resilience to multiple parasites. This practice can also increase genetic diversity and consequently enhance resilience to abiotic and biotic stressors, capacity for adaptive responses, provisioning of ecosystem services, and stability in both the short term and the long term [40,41,43,119]. Alternatively, collecting from large populations experiencing similar environments [41,120,121] may provide comparable benefits of increasing neutral and adaptive genetic variation, as well as phenotypic and functional trait variation, without the risk of outbreeding depression. Further, increasing seeding/planting frequency [121,122] can minimize sampling effects resulting from single collection events and/or propagation methods, and thereby further diversify sources temporally and/or spatially to enhance restoration success. Ultimately, co-design of restoration efforts in an experimental framework, as illustrated here involving resource management agencies, academic scientists, non-governmental conservation organizations and hatcheries/growers, is needed to inform the development of adaptive and feasible management practices that enhance restoration success in a range of environmental contexts.

Data accessibility. Data are available from BCO-DMO (https://doi.org/10.26008/1912/bco-dmo.883570.1) [123].

The data are provided in electronic supplementary material [124].

Authors' contributions. T.C.H.: data curation, formal analysis, investigation, methodology, supervision, validation, writing—original draft, writing—review and editing; J.H.G.: funding acquisition, project administration, resources, supervision, writing—review and editing; E.G.S.: data curation, funding acquisition, project administration, resources, writing—review and editing; P.D.B.: data curation, methodology, resources, writing—review and editing; L.M.P.: data curation, methodology, visualization, writing—review and editing; R.S.: data curation, methodology, visualization, writing—review and editing; G.M.: data curation, methodology, visualization, writing—review and editing; W.S.K.H.: data curation, project administration, resources, writing—review and editing; H.K.: data curation, resources, writing—review and editing; M.C.M.: funding acquisition, methodology, project administration, resources, writing—review and editing; A.R.H.: conceptualization, funding acquisition, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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