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Environmental gradients shape the combined effects of multiple parasites on oyster hosts in the northern Gulf of Mexico

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ABSTRACT: Parasites can shape population, community, and ecosystem dynamics, especially if their hosts play a key role in the ecosystem. Multiple parasites frequently co-infect hosts that alter disease dynamics via a variety of mechanisms. Further, abiotic and biotic factors often differentially affect hosts and their associated parasites, with direct and/or indirect effects on disease prevalence and intensity. We conducted a field survey of eastern oyster *Crassostrea virginica* populations in the northern Gulf of Mexico to examine spatial patterns in parasite species richness of this foundation species. We assessed whether environmental factors and/or host characteristics best predicted prevalence and intensity of the most common micro- (*Perkinsus marinus*; Dermo) and macro-parasites (boring sponges *Cliona* spp., mud blister worms *Polydora websteri*) in the region. The number of parasite species infecting each host (i.e. species richness) was spatially homogeneous due to variation in the predominant factors underlying the prevalence and intensity of each species. These factors were host density, tidal elevation, and temperature for Dermo; tidal elevation and host size for boring sponges; and salinity for mud blister worms. Host condition depended on abiotic and biotic factors, including tidal elevation (intertidal > subtidal) and prevalence of both Dermo (positive relationship) and boring sponges (negative relationship). Abiotic influences on oysters and parasites, and multiple parasite effects, produced an unexpected positive correlation between host condition and Dermo prevalence. Thus, predicting the likelihood of disease outbreaks and assessing the long-term health of host populations requires consideration of the combined effects of multiple parasites, abiotic conditions, and biotic factors.

KEY WORDS: *Crassostrea virginica* · Host–parasite dynamics · Co-infection · Marine pathogens · Infectious disease · Microparasites · Macroparasites

1. INTRODUCTION

Parasites occupy as many as 75% of the trophic links in ecological communities (Lafferty et al. 2006) and can strongly influence community structure and ecosystem functioning, particularly if their host species play a foundational, keystone, or engineering role in the ecosystem (e.g. Anderson et al. 2004, Burge et al. 2014). Host–parasite dynamics depend on a variety of abiotic and biotic factors, creating spatial variation in the abundance of micro- and macroparasites at multiple scales (e.g. Jokela & Lively 1995, Byers et al. 2008, Seabloom et al. 2010). In many cases, multiple environmental factors (e.g. temperature, salinity, and pH in aquatic systems) may underlie spatial variation in parasite abundance,

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challenging efforts to explain host–parasite dynamics. Further, host population characteristics, such as density, age structure, and size structure, can interact with environmental conditions to affect parasite prevalence (i.e. proportion of host population infected) and intensity (i.e. concentration per infected host). Thus, understanding and predicting parasite patterns and disease outbreaks in a time of rapid environmental change requires simultaneous consideration of environmental gradients and host traits.

In addition, parasite diversity comprises a critical yet often overlooked component of food webs and community dynamics, further complicating the development of generalizations about spatial variability in host–parasite dynamics (Lafferty et al. 2008). Multiple parasites frequently co-infect most host species (Pedersen & Fenton 2007, Telfer et al. 2010), and environmental gradients may differentially affect hosts and their associated parasites, confounding interpretation of host responses to individual and multiple parasites. For example, if co-infecting parasite species respond similarly to a physical gradient, changes in overall prevalence and species richness of parasites may vary predictably (Carrol et al. 1990, Lim & Green 1991, Malek & Breitburg 2016). However, if co-infecting parasites respond differently to environmental gradients, within-host parasite community composition may vary less predictably, shifting across environmental gradients (Seabloom et al. 2010). In both cases, the consequences for host condition and susceptibility will depend on both the direct effects of the environmental factor on the host and the indirect effects on the host's parasite community. Consequently, while parasite species richness (i.e. the number of parasite species infecting each host) may be consistent across an environmental gradient, species identity and community composition may differ, potentially resulting in spatially variable net effects on density and condition of the host population. Thus, examining the prevalence and intensity of multiple parasite species represents a key step in understanding host–parasite dynamics across environmental gradients.

As a valuable foundation species in temperate estuaries along the Atlantic and Gulf coasts of North America, the eastern oyster *Crassostrea virginica* (hereafter, 'oyster') supports important fisheries and creates reef habitat that serves as a nursery for a variety of commercially important invertebrates and fishes, provides shoreline stabilization, and improves water quality (Grabowski et al. 2012). The extent of ecosystem functions and services provided by oysters depend in large part on population growth rate and density, which vary considerably along estuarine gradients in salinity and tidal elevation (Kimbro et al. 2009, 2014). For example, freshwater input creates a salinity gradient within estuaries, with lower salinities closer to land and higher salinities closer to sea. The benefits of lower salinity for oysters include potential inhibition of predators (Kimbro et al. 2017, Pusack et al. 2018) or parasites (Powell et al. 1992), but extremely low salinities can increase oyster mortality by inhibiting osmoregulation (La Peyre et al. 2013). Similarly, oyster safety may also vary across tidal elevational gradients, as intertidal, but not subtidal, oysters may experience a refuge from predators (Johnson & Smee 2014) and parasites (La Peyre et al. 2018) that cannot tolerate air exposure, increased temperature, or elevated carbon dioxide levels in the high intertidal. Alternatively, the benefits of elevation could operate in the reverse direction: oysters in the intertidal may be more susceptible to parasitism as a result of compromised immune response and generally poor condition due to the effects of multiple abiotic stressors in the high intertidal (Allen & Burnett 2008).

Although some studies to date have focused on the effects of multiple parasites on oyster populations (Aguirre-Macedo et al. 2007) in an experimental setting or with selectively bred lines (Wargo & Ford 1993, Proestou et al. 2016, Malek & Byers 2017), oysters on natural reefs are regularly exposed to and infected by a variety of micro- and macro-parasite species simultaneously (Ford & Tripp 1996, Pagenkopp Lohan et al. 2016), each of which may respond differently to physical gradients and spatial heterogeneity in host density. To date, whether parasite species richness varies across dominant estuarine gradients of salinity, temperature, tidal elevation, and host population density remains unknown, and which combination of abiotic and biotic factor(s) best predicts the prevalence and intensity of individual parasite species, as well as oyster host condition, merits further investigation.

To address this gap, we surveyed oyster host populations in 2 estuaries in the northern Gulf of Mexico. We focused on 3 important oyster parasite taxa: the microparasite *Perkinsus marinus*, causative agent of the disease Dermo (hereafter, 'Dermo'), and 2 macroparasites: boring sponges (*Cliona* spp., hereafter, 'boring sponges') and mud blister worms *Polydora websteri*. Although boring sponges and mud blister worms do not feed on oyster tissue, and thus do not necessarily fit classical definitions of parasitism, they live on the oyster at some cost to the oyster's fitness; for simplicity, we refer to them as 'parasites' here-

after (Lafferty et al. 2008). We first examined broad spatial patterns in prevalence (i.e. proportion of host population infected) and intensity (i.e. parasite concentration per infected host) of each parasite species on intertidal and subtidal oyster reefs that varied in distance from fresh water and thus water salinity. We then assessed whether host characteristics (i.e. density and size) and environmental factors known to affect both the oyster host and its parasites (i.e. salinity, temperature, and tidal elevation) best predicted prevalence and intensity of each parasite species. Finally, we examined oyster condition to determine which abiotic (salinity, temperature, and tidal elevation) and biotic (parasite prevalence and intensity) factors were most closely associated with the host population, and thus predict the combined effects of multiple parasites on oyster populations in the northern Gulf of Mexico.

2. MATERIALS AND METHODS

2.1. Study system

We studied 2 estuaries in the northern Gulf of Mexico, Florida, USA: Apalachicola Bay and Ochlockonee Bay. Apalachicola Bay, a large, shallow estuary (400 km^2, average depth 1.9 m) with a 50 000 km^2 watershed, originates in northeastern Georgia (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m612p111_supp.pdf); Apalachicola River is the primary source of fresh water to Apalachicola Bay (Mortazavi et al. 2000) and is thus the main driver of salinity gradients within the estuary (Livingston et al. 2000). Ochlockonee Bay, located 30 km northeast of Apalachicola Bay, is a small, shallow estuary (25 km^2, average depth 1.0 m) with a 6500 km^2 watershed that originates in southwestern Georgia; Ochlocknee River is the main source of fresh water to Ochlockonee Bay (Kaul & Froelich 1984), and river flow thus primarily determines water salinity in the bay (Kimbro et al. 2017).

Both bays have subtidal and intertidal oyster populations, with oyster reefs covering approximately 13% of Apalachicola Bay (Kimbro et al. 2017). Historically, oyster populations and the associated fishery in Apalachicola Bay have remained relatively stable (Zu Ermgassen et al. 2012), despite dramatic decreases in oyster abundance, significant losses of reef habitat, and precipitous declines of the oyster fishery worldwide (Lotze et al. 2006, Beck et al. 2011). However, oyster reefs and commercial harvest in Apalachicola Bay decreased dramatically in 2012, with adult oyster (i.e. shell height >25 mm) biomass declining 80% and legally harvestable stock declining 67% on major commercial reefs (FFWCC 2013). Consequently, host population density was relatively low at some sites during this study.

2.2. Parasite species

2.2.1. *Perkinsus marinus* (Dermo)

P. marinus, a protozoan endoparasite that develops and proliferates within host tissues after ingestion by filter-feeding oysters, causes Dermo disease. The effects of Dermo on its oyster host (reviewed by Paynter 1996) include decreased individual and population growth, reproduction, and condition (Crosby & Roberts 1990, Paynter & Burreson 1991, Dittman et al. 2001), which have resulted in mass mortality events along the US Atlantic and Gulf coasts (Burreson & Ragone Calvo 1996, Soniat 1996). Past studies have linked the prevalence and intensity of Dermo infection to a variety of abiotic and biotic factors (Burreson & Ragone Calvo 1996, Oliver et al. 1998, Lenihan et al. 1999). Both, high salinities (Crosby & Roberts 1990, Burreson & Ragone Calvo 1996, Bushek et al. 2012) and high summer temperatures (Bushek et al. 2012, Petes et al. 2012, Bidegain et al. 2017), have been associated with greater prevalence and higher intensity of Dermo in field and laboratory studies. Seasonal changes in oyster–Dermo dynamics associated with temperature variation can also affect the relative importance of host density and density-dependent transmission in determining the persistence of Dermo and the risk of future epizootics (Bidegain et al. 2017).

2.2.2. *Cliona* spp. (boring sponges)

Boring, clionaid sponges are macroparasites that affect commercial shellfish worldwide by reducing oyster growth, recruitment, and condition (Barnes et al. 2010, Carroll et al. 2015). Historically prevalent along the Atlantic (Leidy 1889) and Gulf (Hopkins 1956) coasts, boring sponges compromise oyster shell integrity and decrease marketability of infected hosts (Carver et al. 2010). In addition, oyster hosts may divert energy to shell deposition in response to boring sponge parasites, thus decreasing growth and condition (Carroll et al. 2015), as well as increasing susceptibility to predators (Lindquist 2011, Coleman 2014) and possibly to additional parasitic infections (Alagarswami & Chellam 1976). Environmental fac-

tors influencing prevalence and intensity of boring sponge infection include salinity (Hopkins 1956, Stubler et al. 2017), habitat type (i.e. subtidal versus intertidal; Lindquist 2011), and temperature (Miller et al. 2010). Whereas sponge-colonized hosts occur more frequently at higher salinities and almost exclusively on subtidal reefs (Hopkins 1956, Lindquist 2011), the effects of host density and size on boring sponge infestation remain equivocal (Rosell et al. 1999, Carroll et al. 2015).

2.2.3. *Polydora websteri* (mud blister worm)

The mud blister worm, a shell-boring polychaete, inhabits the shells of oysters and many ecologically and commercially important bivalves (Lauckner 1983, Wargo & Ford 1993). Prevalent along the Atlantic and Gulf coasts (Hopkins 1958, Blake 1969), mud blister worm larvae settle onto the outer shell of their hosts and construct a mud-lined burrow, using the oyster as protection. This macroparasite causes the host to invest energy in shell repair, secreting a protective layer and diverting energy from growth and reproduction, which may result in reduced internal cavity volume, decreased size, and poor condition index (Wargo & Ford 1993, Dunphy et al. 2005), as well as decreased marketability. Past studies have associated mud blister worm abundance with a variety of environmental factors, including salinity (Lauckner 1983), temperature (Lauckner 1983), and tidal height/air exposure (Handley & Bergquist 1997, Royer et al. 2006), as well as host density (Zajac 1991). Efforts to eradicate the worm parasite in aquaculture have focused on hypo- and hyper-salinity treatments (Dunphy et al. 2005), indicating that intermediate salinities may be optimal for mud blister worms.

2.3. Field survey

Prior to the field survey, we partitioned commercial and non-commercial oyster reefs in Apalachicola Bay and Ochlockonee Bay into 3 zones based on their relative distance from the primary source of freshwater into the system (Fig. S1 in the Supplement; see Kimbro et al. 2017 for details). Because the Apalachicola River is centrally located in Apalachicola Bay and water from the Gulf of Mexico primarily enters the bay from the east and exits the bay to the west, we further divided these distance zones into east versus west of the Apalachicola River (Fig. S1). We used this spatially stratified sampling approach because we lacked prior information on salinity, temperature, or other water quality parameters, and we wanted to ensure that our random samples would capture the full range of estuarine conditions. The distance of each reef from the mouth of the river was calculated using a Euclidean distance function, and reefs were assigned to a zone based on both their relative position to the river (i.e. east Apalachicola or west Apalachicola) and their distance from river input (i.e. close, mid, or far; Fig. S1). In July 2015, we sampled 3 randomly selected subtidal reefs within each zone, as well as intertidal reefs where present (i.e. only in the east-mid and west-far zones of Apalachicola Bay, and in the mid and far zones of Ochlockonee Bay), to estimate oyster abundance and size distribution (see Table S1 in the Supplement for a complete list of sites and sampling dates). For subtidal reefs, we positioned the boat directly above the center of the reef and extended 4 transects (20 m each) from the boat at right angles from each other (i.e. 0, 90, 180, and 270°). Along each transect, we collected the entire contents of a 0.25 m^2 quadrat at the 5, 10, 15, and 20 m marks to obtain spatially balanced samples of oyster density and size structure. For intertidal reefs, we sampled 2 quadrats per reef, 'low' (located at the low water level) and 'high' (2 m above the low transect) quadrats centered along a 20 m transect on each reef.

Samples were collected in mesh bags and immediately placed on ice. At the laboratory, we determined total oyster biomass for each quadrat prior to sample processing. We measured the first 100 individual oysters and then counted the remaining number of juvenile (<25 mm) and adult (>25 mm) oysters in each quadrat. In addition, we quantified and measured all 'new' gapers (i.e. recently dead oysters that still contained soft tissue) and counted all 'old' gapers (i.e. intact shells that did not contain any soft tissue). If available, 2 adult oysters from each quadrat were placed immediately on ice and stored at −80°C for disease analyses.

To assess water properties and provide environmental context for field survey data, we conducted monthly conductivity-temperature-depth (CTD) profiles of the water column using a Sea-Bird Electronics SBE 19plus V2 SeaCAT Profiler CTD at 30 and 19 stations across zones in Apalachicola Bay and Ochlockonee Bay, respectively. CTD monitoring began in September 2014 and January 2015 for Apalachicola Bay and Ochlockonee Bay, respectively; for each station, we calculated the median daily salinity and temperature across depths, and then used daily medians to generate monthly means.

2.4. Microparasite prevalence and intensity

To assess prevalence and intensity of Dermo infection in adult oysters from the 2015 field survey, we used a quantitative polymerase chain reaction (qPCR) assay. For each oyster, we collected and weighed a sample of gill and mantle tissue (~25 mg) to extract DNA using an Omega Bio-Tek E-Z 96® Tissue DNA Kit. The samples were analyzed using a modified version of the Gauthier et al. (2006) qPCR protocol on a Bio-Rad CFX96™ Real-Time System and Bio-Rad CFX Manager software (version 3.1). Each reaction contained 1 µl template DNA, 3.5 µl water, 5 µl Sso-Advanced™ Universal Probes Supermix (Bio-Rad), and 0.5 µl 20× primer/probe master mix, including 18 µM of each PMAR primer and 5 µM of the PMAR TaqMan® MGB probe (Gauthier et al. 2006). The cycling conditions consisted of an initial denaturation step at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 56°C for 30 s. We used gBlocks® (gene fragments containing the target region from *P. marinus*; Integrated DNA Technologies) to develop a standard curve to assess infection levels and extracted DNA from a culture of *P. marinus* with a known quantity of cells for use as a positive control. All standards, samples, and positive and negative controls were run in duplicate; if samples differed by >1 Cq, they were rerun to confirm infection intensity. In addition, a subset of oyster samples was processed using standard Ray's fluid thioglycollate medium (RFTM) assays (Ray 1966) to confirm the presence/absence of hypnospores and quantify infection intensity in gill and mantle tissue. In no case did we get a positive result using the qPCR assay for a sample that lacked hypnospores, indicating that while the reported detection limit of the qPCR assay is lower than that of the RFTM assay, we found no evidence that this resulted in false positives. In addition, a qualitative comparison of hypnospore counts using the RFTM assay and cell densities using the qPCR assay indicated that sample intensities were consistent using both methods.

2.5. Macroparasite prevalence and intensity

For each oyster, we measured a suite of characteristics to assess life stage, size, and condition index, including shell height of adults, total wet mass, wet and dry shell mass, and wet and dry tissue mass. In addition, we assessed disease prevalence and infection intensity for boring sponges and mud blister worms. To quantify boring sponge infection intensity, we measured the lengths of the top and bottom valves of each oyster (i.e. shell height), photographed each valve, and measured the total proportion of shell area affected by boring sponges using ImageJ (Abràmoff et al. 2004; Fig. S2 in the Supplement). Intensity was calculated as (boring sponge area / oyster shell area) × 100 for the top and bottom valves. In addition, we quantified the number of mud blisters present on the top and bottom valves of each oyster to estimate mud blister worm prevalence and intensity, with intensity equal to (mud blister count / oyster shell area). In a preliminary study of a subset of field survey samples, we found that mud blister count was a good proxy for mud blister area ($R^2 = 0.51$, $p < 0.001$), so we present mud blister count, which is much easier and faster to quantify than mud blister area and thus more useful and relevant to managers and practitioners as a measure of intensity. To relate parasite prevalence and intensity to oyster condition index, we calculated condition index as (dry tissue weight × 100 / dry shell weight).

2.6. Statistical analysis

To examine spatial patterns in parasite species richness within oyster populations in our focal estuaries, we first examined whether the number of parasite species infecting each host varied across environmental gradients. Given the multivariate nature of our dataset, we then used non-metric multidimensional scaling (nMDS) to visualize the data and identify major patterns of variability. We then tested individual hypotheses (a frequentist approach) and then a separate model selection analysis (an information-theoretic approach) to assess the relative importance of abiotic and biotic factors in determining the prevalence and intensity of each parasite species, as well as host condition index.

First, to identify any broad spatial patterns in parasite prevalence across oyster reefs in Apalachicola Bay and Ochlockonee Bay, we used generalized linear models to assess whether parasite species richness (ranging from 0–3) varied between estuaries (Apalachicola versus Ochlockonee), across tidal elevations (intertidal versus subtidal), or with distance from freshwater input. To account for differences in size between estuaries (following the approach of Kimbro et al. 2017), we calculated the Euclidean distance of each site from the river mouth (Apalachicola River in Apalachicola Bay and Ochlockonee River in Ochlockonee Bay), and then standardized the distances (i.e. proportional distance = distance of site

from the river / distance of site farthest from the river within each estuary). This standardization of distance was conducted separately for each region (i.e. east versus west) of Apalachicola Bay. In addition, we examined the relationship between each pairwise combination of oyster parasites (i.e. boring sponges versus mud blister worms, boring sponges versus Dermo, and mud blister worms versus Dermo) for both prevalence and intensity to assess whether infection/colonization by one species was related to that by another species, and whether this varied depending on species identity (Wargo & Ford 1993).

Second, we used nMDS with the 'vegan' package in R 3.0.2 (Oksanen et al. 2016) to relate spatial patterns in parasite prevalence and intensity across oyster reefs (see Section 3.1 and Fig. 1) to abiotic and biotic explanatory variables. Specifically, we examined the relative importance of environmental (salinity, temperature, distance from freshwater input, tidal elevation, and estuary) and biological (oyster host density and size) factors in explaining parasite prevalence and intensity. The ability of the nMDS projection to preserve the ranked multivariate distances between samples was expressed as R^2 (R^2 is equal to 1 − nMDS stress, which is also commonly reported). To interpret the axes, we used a permutation-based approach to determine which variable(s) were significantly related to each axis ($p < 0.05$), plotting them as vectors with direction and length representing the correlation between axes and variables.

Third, we took a hypothesis-testing approach to determine which abiotic and biotic factors were related to prevalence and intensity of each parasite species. For parasite prevalence, we only tested for relationships with those variables significantly related to 1 of the nMDS axes. However, a limitation of the nMDS analysis of parasite intensity is that it excluded sites without all parasite species (i.e. if prevalence equals 0, then intensity is undefined), so for this response variable, we explored all possible environmental and biological explanatory variables in our hypothesis-testing approach. In both cases, we controlled for false discovery rate using the Benjamini-Hochberg procedure (Benjamini & Hochberg 1995). For these analyses, we used generalized linear models with the 'lme4' package in R 3.0.2 (Bates et al. 2015). Because we sampled in mid-summer, we used early summer salinity and temperature (i.e. average of June and July 2015) as predictors of parasite prevalence and intensity in the models.

Fourth, we used model selection to identify which combination of environmental and biological variables best predicted prevalence and intensity of each parasite species. Because of limited replication resulting from the recent collapse of the oyster population in this region (FFWCC 2013), comparisons included only a null model, and models with each single fixed factor. For parasite prevalence, only factors identified as significant predictors in the univariate analyses (see Section 3.2 and Table S2a) were included in model selection. Prevalence data were analyzed using logistic regression, with a binomial error distribution and a logit link function. For parasite intensity, we included all possible environmental and biological explanatory variables to assess the relative predictive power of each factor since hypothesis testing did not identify any significant relationships, due primarily to a lack of power. For these analyses, models were constructed in R 3.0.2 using the 'lme4' package (Bates et al. 2015), fit using maximum likelihood, and compared using Akaike's information criterion corrected for small sample size (AIC_c) to identify the most parsimonious model(s) using the 'bbmle' package (Burnham & Anderson 2002, Bolker & R Development Core Team 2014). Models were ranked according to Akaike weight (w_i, model relative likelihood normalized by the sum of all model relative likelihoods), which indicates the probability that a given model is the best model (Burnham & Anderson 2002, Johnson & Omland 2004).

Lastly, to examine how host condition index related to parasite prevalence, we again used a hypothesis-testing approach followed by model selection. Although condition index may vary seasonally and fluctuate depending on reproductive stage (Dittman et al. 2001), we sampled within a 2 wk window during the summer following spring gametogenesis and spawning and prior to the fall reproductive period, minimizing the effects of seasonal variation and reproductive status on host condition. In addition, we observed no noticeable differences in the ripeness of oysters collected from different sites and across tidal elevations during processing (T. C. Hanley pers. obs.). In the first set of univariate analyses, we included individual parasite prevalence and host population characteristics (i.e. oyster density and size), as well as environmental factors because of clear spatial variation in parasite prevalence patterns across estuaries, tidal elevations, and distances from fresh water (i.e. across a salinity gradient) (see Section 3.2). Models and analyses used the same approach and criteria described above, except comparisons included a null model, models with each single fixed factor, and all additive combinations of parasite prevalence to examine the combined effects of multiple parasites on host condition. None of the models included any

combinations of co-infection (e.g. oysters with boring sponges and mud blister worms versus oysters with Dermo and boring sponges) because of the low and variable proportion of oysters with various iterations of multiple parasite species across tidal elevation (a factor in the model). Because one of the best models for condition index included the additive effects of Dermo prevalence and boring sponge prevalence (see Section 3.4), we used partial regression analysis to assess how well each of these parasites independently predicted host condition index (Quinn & Keough 2002).

3. RESULTS

3.1. Parasite species richness

Our survey of oyster parasite species richness (including Dermo, boring sponges, and mud blister worms) showed no clear spatial pattern in the number of parasite species infecting each host between estuaries ($F_{1,17}$ = 0.68, p = 0.42), across tidal elevations ($F_{1,17}$ = 2.55, p = 0.13; Fig. 1A), or with relative distance from freshwater input ($F_{1,17}$ = 0.00, p = 0.99; Fig. 1B). However, the relative prevalence of each parasite species differed between tidal elevations (Fig. 1A): Dermo prevalence (i.e. proportion of oysters infected with *Perkinsus marinus*) was 4 times greater on intertidal than subtidal reefs, whereas boring sponge prevalence was 2 times higher on subtidal than intertidal reefs, and mud blister worm prevalence was similar across reef types. In addition, spatial patterns of prevalence differed among parasite species, with Dermo prevalence increasing and mud blister worm prevalence decreasing with respect to relative distance from fresh water, and boring sponge prevalence peaking at intermediate distances from fresh water (Fig. 1C–E). In pairwise comparisons of parasite species, prevalence or intensity of one species was unrelated to prevalence or intensity of another species (r < 0.5 and p > 0.05 for all comparisons; Fig. S3 in the Supplement). However, almost 70% of the oysters sampled were infected by multiple parasites, with 10% of oysters having Dermo and boring sponges, 20% of oysters having Dermo and mud blister worms, 30% of oysters having boring sponges and mud blister worms, and 8% of oysters having all 3 parasites.

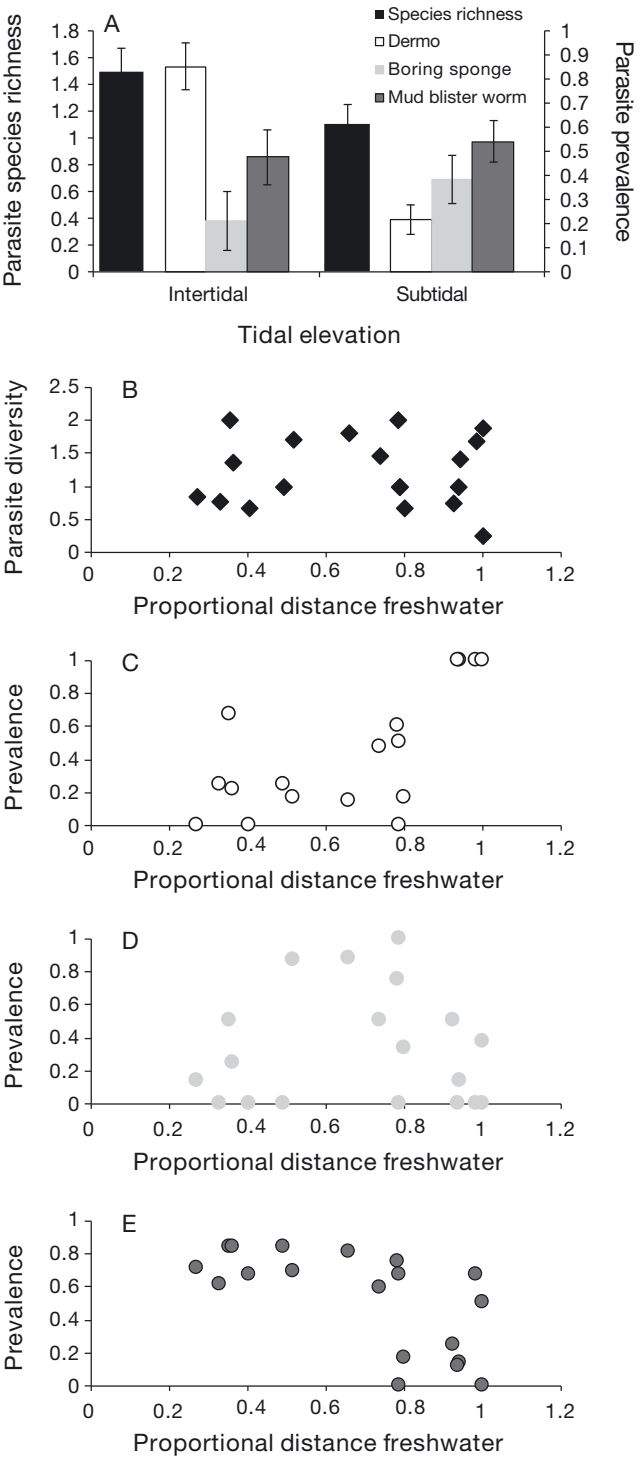

Fig. 1. (A) Mean ± SE parasite species richness (left *y*-axis) of intertidal and subtidal oysters collected during the field survey, and mean ± SE prevalence (i.e. proportion of infected hosts) of each parasite species (right *y*-axis). Relationships between distance from freshwater input and (B) parasite species richness, (C) Dermo prevalence, (D) boring sponge prevalence, and (E) mud blister worm prevalence. Proportional distance from fresh water is calculated as (distance of each reef) / (distance of the farthest reef) within each estuary to standardize comparisons across Apalachicola Bay and Ochlockonee Bay. Each point represents a replicate reef

3.2. Parasite prevalence

The nMDS identified clear differences in parasite prevalence across intertidal and subtidal oyster reefs (i.e. tidal elevation), with both environmental (salinity) and biological (host density) factors influencing the proportion of infected/colonized hosts and the identity of parasite species (Fig. 2). In particular, sites with high Dermo prevalence were primarily intertidal reefs located farther from freshwater input and characterized by greater host densities and moderate to high salinities. In contrast, sites with high mud blister worm prevalence were mostly subtidal reefs located closest to freshwater input and characterized by low salinities and intermediate host densities, whereas sites with high boring sponge prevalence were mainly subtidal reefs located mid- to far-distance from freshwater input, and characterized by moderate salinities and low to intermediate host densities.

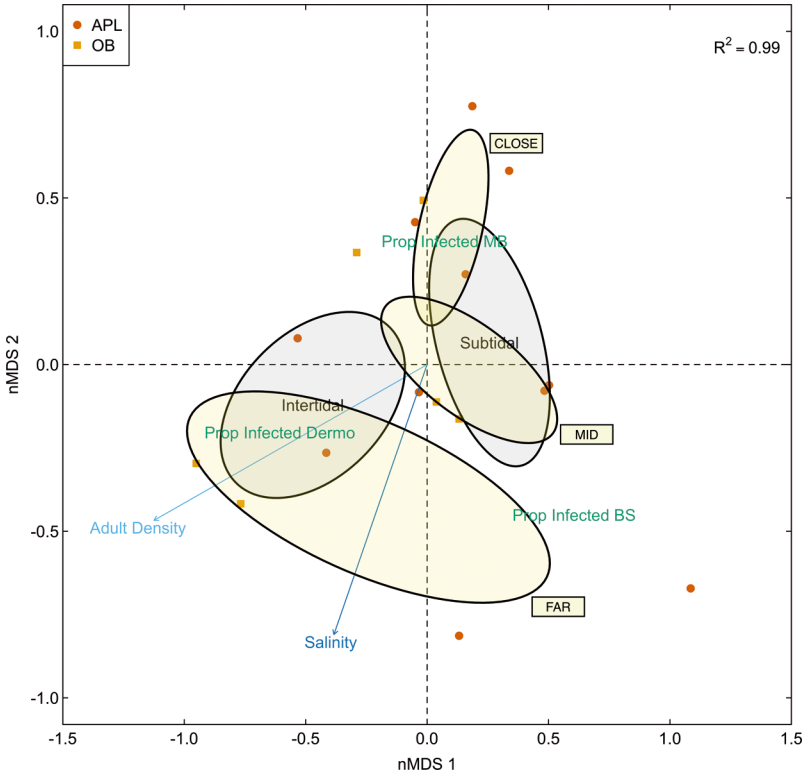

Fig. 2. Non-metric multidimensional scaling (nMDS) plot depicting prevalence of the microparasite (Dermo), and the macroparasites (BS: boring sponges, MB: mud blister worms) on oyster reefs in Apalachicola Bay (APL) and Ochlockonee Bay (OB) with significant explanatory variables (abiotic: salinity; biotic: host density) correlated to the axes. For tidal elevation (intertidal and subtidal; grey ellipses) and zone (close, mid, and far from freshwater input; yellow ellipses), ellipses include 95% confidence intervals, with non-overlapping circles indicating significant differences. Each point represents a replicate reef

In almost every case, the environmental and biological factors identified by nMDS were significant predictors of parasite prevalence (Table S2A in the Supplement); thus, we used model selection to identify the best predictor(s) of prevalence for each species (Table S2B). For Dermo prevalence, the best models included host density (ΔAICc = 0, w_i = 0.57) and tidal elevation (ΔAICc = 0.6, w_i = 0.43) as important predictors, with prevalence positively associated with host density (Fig. 3B), and a greater proportion of infected oysters on intertidal than subtidal reefs (Fig. 3A). For boring sponge prevalence, the best models again included tidal elevation (ΔAICc = 0, w_i = 0.64) and oyster density (ΔAICc = 2.2, w_i = 0.21), but with a greater proportion of infected oysters on subtidal than intertidal reefs (Fig. 3C), and prevalence negatively associated with host density for boring sponges (Fig. 3D). For mud blister worm prevalence, the best model included only salinity (w_i = 0.99), with the proportion of adult oysters with mud blisters decreasing as salinity increased (Fig. 3E).

3.3. Parasite intensity

In contrast to parasite prevalence, nMDS did not identify large differences between parasite intensities, with oyster density as the only biotic factor and tidal elevation as the only abiotic factor influencing infection intensity. In addition, sites with higher concentrations of Dermo and/or greater mud blister area were generally more similar to each other than to sites with high boring sponge percent cover (Fig. S4 in the Supplement). In almost all cases, our hypothesis testing approach found weak or no relationships between parasite intensity and measured abiotic and biotic factors (Table S3A in the Supplement). However, Dermo intensity was related to tidal elevation and temperature, as well as estuary.

To further explore the relative importance of environmental and biological variables in influencing infection/colonization intensity, we used model selection to identify the best predictor(s) of intensity for each species (Table S3B). For Dermo intensity, the

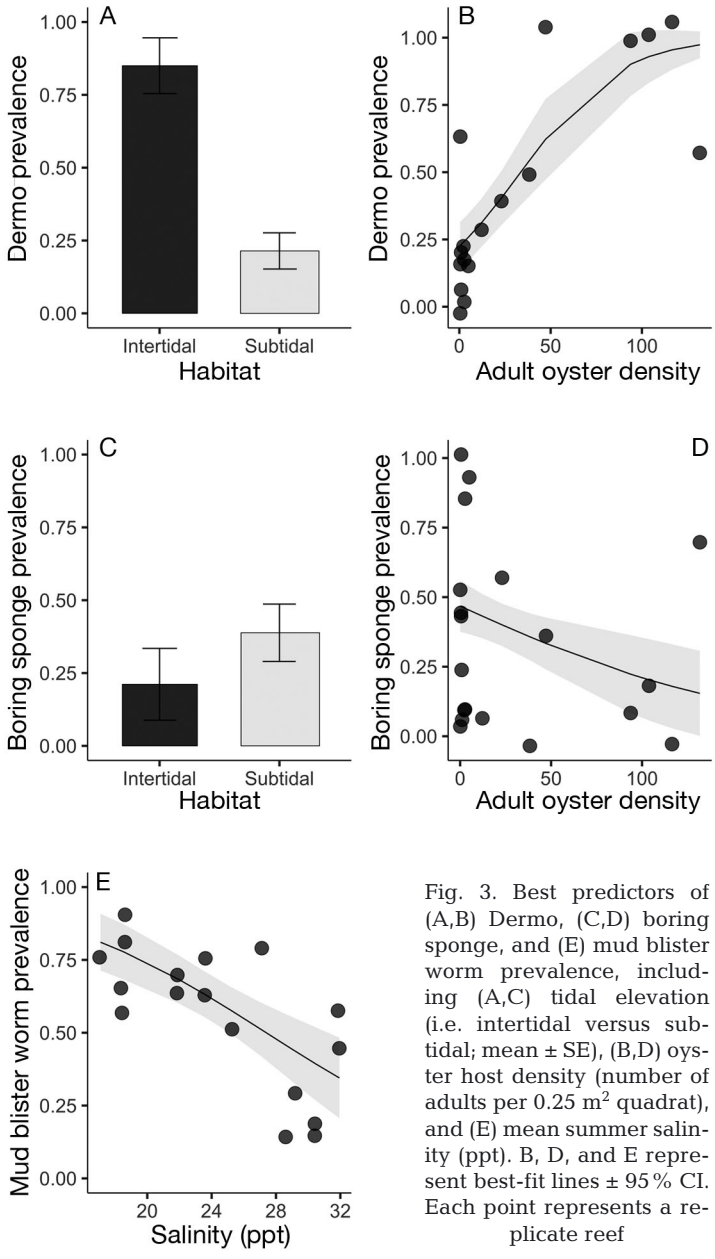

Fig. 3. Best predictors of (A,B) Dermo, (C,D) boring sponge, and (E) mud blister worm prevalence, including (A,C) tidal elevation (i.e. intertidal versus subtidal; mean ± SE), (B,D) oyster host density (number of adults per 0.25 m^2 quadrat), and (E) mean summer salinity (ppt). B, D, and E represent best-fit lines ± 95% CI. Each point represents a replicate reef

best models included tidal elevation (ΔAICc = 0, w_i = 0.49), temperature (ΔAICc = 1.7, w_i = 0.21), and estuary (ΔAICc = 1.7, w_i = 0.21), with higher intensities on intertidal than subtidal reefs (Fig. 4A) and infection intensity positively associated with increasing water temperature (Fig. 4B), as well as slightly greater intensities in Ochlockonee Bay than Apalachicola Bay (mean ± SE: Ochlockonee 5.50 ± 0.27; Apalachicola 4.18 ± 0.34 log[cells *Perkinsus* / g oyster tissue]). For boring sponge intensity, the best models included oyster size (ΔAICc = 0, w_i = 0.31) and tidal elevation (ΔAICc = 2.0, w_i = 0.12), as well as the null model (ΔAICc = 0.3, w_i = 0.27). Boring sponge intensity was negatively associated with oyster size; a smaller percentage of the shell was colonized by boring sponges as oyster shell length increased (Fig. 4C). For mud blister worm intensity, the best models included salinity (ΔAICc = 0, w_i = 0.31), estuary (ΔAICc = 2.3, w_i = 0.13), and temperature (ΔAICc = 2.3, w_i = 0.13), as well as the null model (ΔAICc = 1.4, w_i = 0.20). Mud blister worm intensity related negatively to salinity, with the number of mud blisters per oyster shell area decreasing with increasing salinity (Fig. 4D), in contrast to a positive association between mud blister intensity and temperature. In addition, mud blister worm intensity was slightly higher in Ochlockonee Bay than Apalachicola Bay (mean ± SE: Ochlockonee 0.23 ± 0.05; Apalachicola 0.15 ± 0.03 mud blister count / oyster shell area mm^2).

3.4. Host condition

Hypothesis testing identified tidal elevation and oyster density, as well as Dermo prevalence and boring sponge prevalence, as significant predictors of host condition index (Table S4A in the Supplement). Based on model selection (Table S4B), the best models for oyster condition index included tidal elevation (ΔAICc = 0, w_i = 0.53), with better overall condition of oysters from intertidal reefs than from subtidal reefs (Fig. 5A), and the additive effects of Dermo prevalence and boring sponge prevalence (ΔAICc = 1.4, w_i = 0.27). In the partial regression analysis, the residuals of boring sponge prevalence and Dermo prevalence were negatively and positively associated with the residuals of oyster condition index, respectively (boring sponge $F_{1,15}$ = 8.33, R^2 = 0.36, p = 0.011; Dermo $F_{1,15}$ = 7.50, R^2 = 0.33, p = 0.015; Fig. 5B,C), indicating that each parasite independently explained a portion of the variation in host condition after accounting for variation resulting from the other parasite.

4. DISCUSSION

In this study, the prevalence of 3 common oyster parasites varied differently along 2 dominant estuarine physical gradients, i.e. tidal elevation and dis-

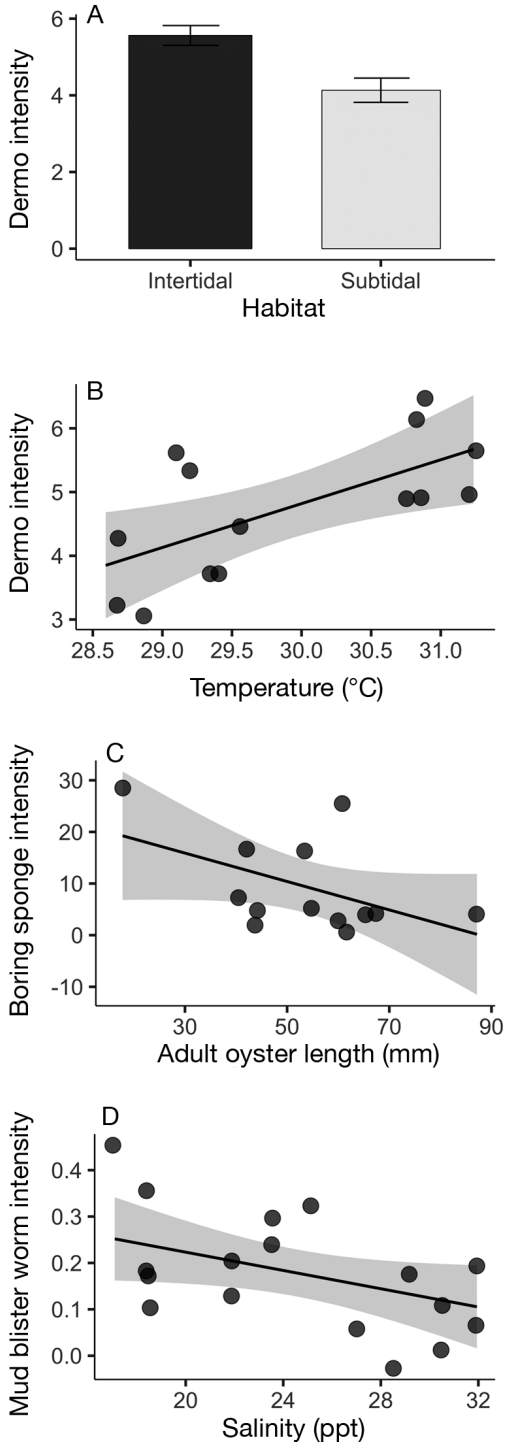

Fig. 4. Best predictors of parasite intensity for (A,B) Dermo (log cells *Perkinsus* g^{-1} oyster tissue), (C) boring sponges (% oyster shell area colonized), and (D) mud blister worms (number mud blisters per oyster shell area mm^2), based on (A) tidal elevation, (B) early summer water temperature (°C), (C) oyster host size (bottom valve length, mm), and (D) mean summer salinity (ppt). Panel A shows mean ± SE; B–D represent best-fit lines ± 95% CI. Each point represents a replicate reef

tance from freshwater input, resulting in spatial heterogeneity in parasite species identity across reefs, but no differences in the average number of parasite species infecting/colonizing individual oysters. In other words, varying spatial gradients in parasite distribution led to similar parasite species richness across space. This pattern likely reflects variation in the predominant factors underlying the distribution, prevalence, and intensity of each parasite species: host density and tidal elevation best predicted Dermo prevalence, with moderate effects of temperature and tidal elevation on intensity; tidal elevation and host density best predicted boring sponge prevalence, while intensity was associated with host size and tidal elevation; and mud blister worm prevalence and intensity related most strongly to salinity, with a moderate effect of temperature on intensity. Thus, the combined effects of multiple parasites on oyster populations likely depend on the interplay of both environmental and biological factors. This result may explain why host condition index, which depended in part on tidal elevation, also related to multiple parasite species, with additive effects of Dermo prevalence and boring sponge prevalence on oyster condition.

While prevalence and intensity of each parasite species has been linked with salinity (e.g. Hopkins 1956, Lauckner 1983, Powell et al. 1992), mud blister worms were most strongly associated with distance from freshwater and with salinity in our study. Specifically, mud blister worm prevalence and intensity were highest at intermediate salinities (20–25 ppt) and decreased at higher salinities (>25 ppt). This finding complements previous studies demonstrating greater mortality of mud blister worms under hypo- and hyper-saline conditions (Dunphy et al. 2005). In addition, the consistent effects of distance from fresh water and salinity on mud blister worm prevalence and intensity demonstrates that distance from freshwater input provides a good proxy for salinity in our system. While Dermo prevalence was positively associated with increasing distance from freshwater input and boring sponge prevalence peaked at intermediate distance from fresh water, salinity was not the best predictor of prevalence or intensity for either parasite species. Thus, while freshwater input was associated with Dermo and boring sponge prevalence overall, it was not the primary driver of host–parasite dynamics for either species in this system during the study period.

Tidal elevation (i.e. intertidal versus subtidal) can also affect both oyster and parasite population dynamics (Lenihan et al. 1999, Powers et al. 2009, Geh-

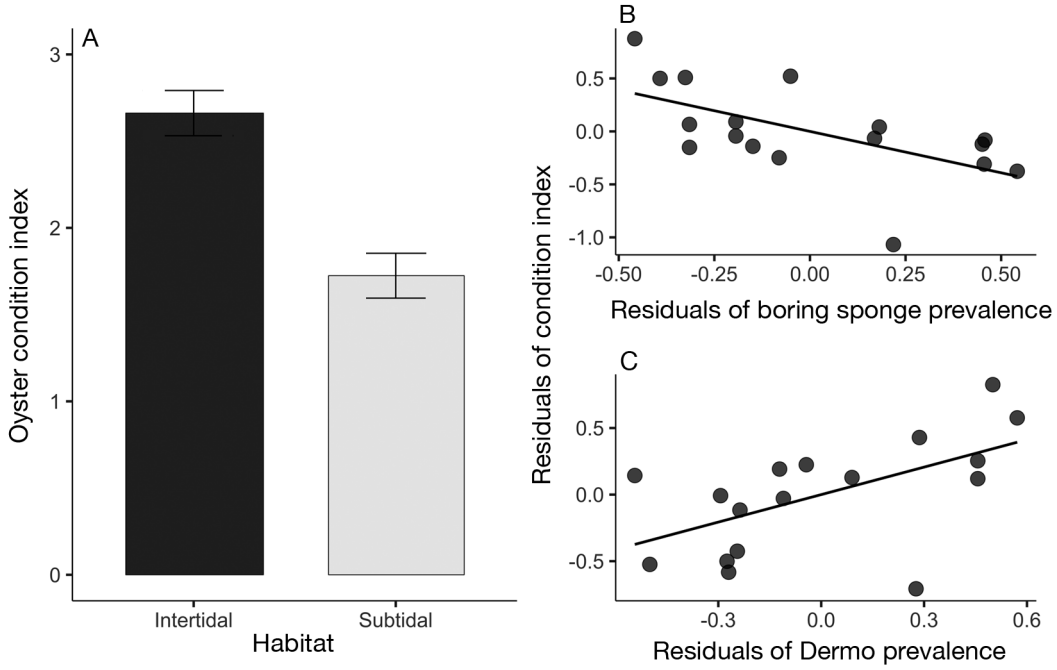

Fig. 5 (A) Mean ± SE oyster condition index (calculated as dry tissue weight × 100 / dry shell weight) on intertidal and subtidal reefs in Apalachicola Bay and Ochlockonee Bay. Also shown are partial regression plots of the residuals of oyster condition index versus the residuals of (B) boring sponge prevalence and (C) Dermo prevalence

man et al. 2017). In our study, we found that the prevalence of each species on intertidal and subtidal oyster reefs depended on parasite identity: Dermo prevalence was much greater on intertidal than subtidal reefs, whereas boring sponge prevalence was somewhat higher on subtidal than intertidal reefs, and mud blister worm prevalence did not differ substantially across tidal elevations. On both intertidal and subtidal reefs, oysters experience multiple environmental stressors, but the identity and magnitude of these factors often vary between reef types, thus favoring parasites with different life histories and environmental niches (Lenihan et al. 1999). For example, oysters on intertidal reefs experience daily air exposure and thus greater variability in temperature, to which boring sponges may be less tolerant than Dermo (Lindquist 2011); this interpretation matches the pattern of higher boring sponge prevalence in oysters from subtidal reefs found in our study.

The higher prevalence of Dermo on intertidal than subtidal reefs at our sites differs from other studies comparing habitat types: a comparison of oyster sanctuaries in North Carolina with intertidal and subtidal reefs reported a generally higher proportion of oysters infected with Dermo at subtidal sites (Powers et al. 2009), whereas a field manipulation in Georgia found no effect of tidal elevation on Dermo prevalence (Malek & Byers 2017). However, examination of the abiotic and biotic factors predicting Dermo prevalence in our study (Fig. 2) suggests that differences in host density may underlie this tidal elevation pattern: oyster density was a strong predictor of Dermo prevalence, with a greater proportion of infected oysters on high density reefs. Our study documented significantly higher densities on intertidal reefs than subtidal reefs, with mean densities of <2 oysters per 0.25 m^2 quadrat at many subtidal sites as a result of the recent collapse of the subtidal oyster fishery in this region (FFWCC 2013, Kimbro et al. 2017). Thus, given the importance of host density in transmission and persistence of Dermo (Bidegain et al. 2017), it is difficult to separate the effects of tidal elevation versus host density on Dermo prevalence in this study. Assessing the relative importance of these factors requires field experiments that manipulate oyster density across intertidal and subtidal reefs, and measure Dermo prevalence and intensity.

In addition to environmental factors, characteristics of the host population predicted several aspects of disease prevalence and intensity. For example, oyster density correlated positively with Dermo prevalence, and oyster size was negatively associated with boring sponge intensity. In contrast, neither was related to mud blister worm prevalence or intensity,

suggesting that changes in oyster density and population size structure as a result of environmental stress and harvest may have little effect on the distribution or abundance of the mud blister worm, making it a relatively resilient parasite, at least within a tolerable salinity range. However, Dermo dynamics may be most influenced by changes in oyster density, which corresponds with observations of high Dermo prevalence and high oyster density on subtidal reefs in Apalachicola Bay historically (100% in 1993–1994, Oliver et al. 1998; >50% in 2007–2008, Petes et al. 2012) compared to relatively low Dermo prevalence and low oyster density (<25% in 2015, this study) following the collapse of the oyster population and fishery in this region.

Low host population density may also have contributed to the relatively weak parasite intensity patterns in our study, with only moderate associations between infection intensity and most abiotic and biotic factors. In general, parasite load was relatively low in this system, with <30% boring sponge percent cover, count and area of mud blister worm constituting <25% of shell area, and light to moderate infection levels of Dermo in gill and mantle tissue, especially compared to historical levels (Oliver et al. 1998). This may explain the absence of a strong relationship between parasite intensity and any abiotic or biotic factor in our study. However, Dermo intensity was higher on intertidal than subtidal reefs, which matches the results of a field manipulation on the Atlantic Coast (Malek & Byers 2017). In addition, Dermo intensity was positively associated with temperature, which is consistent with observed patterns of epizootics concomitant with warming temperatures (Cook et al. 1998, Powell 2017). Similarly, the negative association between mud blister worm intensity and salinity in our study confirms reports of reduced mud blister worm infestation with exposure to high salinities (Dunphy et al. 2005). Boring sponge intensity was negatively associated with oyster length, suggesting that host population size structure may affect oyster–sponge dynamics. While the negative relationship between oyster length and sponge intensity in our field survey contrasts with a previous study documenting a positive relationship between oyster shell area and boring sponge biomass (Carroll et al. 2015), this may be due in part to the relatively low percent cover or the generally smaller size, and therefore younger age, of oysters in our study.

Despite the fact that parasite load was moderate in this system, comparable infection levels in other studies had at least modest effects on oyster condition index (e.g. Crosby & Roberts 1990, Wargo & Ford 1993, Carroll et al. 2015), and the additive effects of Dermo prevalence and boring sponge prevalence were strongly associated with oyster condition index in our study. Tidal elevation also strongly predicted oyster condition index. Interestingly, oyster condition index was positively associated with Dermo infection, which was more prevalent on intertidal reefs, but negatively associated with boring sponge colonization, which was more prevalent on subtidal reefs. Similarly, whereas >75% of intertidal oysters compared to 50% of subtidal oysters were infected with at least 1 parasite, condition index of oysters from intertidal reefs generally exceeded that of oysters from subtidal reefs. In general, greater feeding time and possibly lower stress often result in better condition of subtidal oysters than intertidal oysters (Crosby et al. 1991). Although in some cases condition index does not differ between reef habitats (Mercado-Silva 2005), condition index of intertidal oysters rarely exceeds that of subtidal oysters. However, differences in oyster condition index between intertidal and subtidal reefs in our system may be due to a number of factors, including parasite prevalence, predation risk, resource availability, flow speed, or oxygen availability, many of which likely interact to determine overall health (Lenihan 1999) and may have contributed to the recent collapse of the subtidal oyster fishery in this region (FFWCC 2013, Kimbro et al. 2017).

Understanding the effects of individual parasite species on host populations, as well as how abiotic and biotic factors influence the strength and direction of host–parasite interactions, provides a critical foundation for disease ecology. Yet host–parasite dynamics frequently involve the interaction of multiple parasites within individual hosts (Pedersen & Fenton 2007, Telfer et al. 2010), and thus predicting the likelihood of a disease outbreak or the long-term health of a host population requires consideration of the independent and interactive effects of multiple parasites. We found no differences in overall parasite species richness across oyster reefs; instead, we identified broad spatial patterns in the prevalence of each parasite species related to tidal elevation and salinity (i.e. distance from freshwater input), with almost 70% of oysters co-infected by multiple parasites. The factors determining the prevalence and intensity of each parasite species also varied, indicating that while oysters are frequently exposed to and infected by multiple parasites, the primary burden on the host may result from a single species, depending on which parasite species is best adapted to the local environment, including abiotic conditions and host population pa-

rameters. Whether this pattern is consistent for host–parasite interactions through time (i.e. across seasons; Powell et al. 1992, Wargo & Ford 1993) and in diverse ecosystems merits further investigation, particularly studies manipulating parasite diversity and measuring the independent and interactive effects on host life history and population dynamics.

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