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If you encyst: evidence of parasite escape and host-switching among three co-occurring crabs

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ABSTRACT: Biological invasions influence species interactions around the globe, including host and parasite communities. We evaluated trematode parasite diversity and the potential for host-switching of parasites in 3 co-occurring crabs in the Northeast USA, including 1 native species (*Cancer irroratus*) and 2 non-natives (*Carcinus maenas*, *Hemigrapsus sanguineus*), of which the former represents a historical and the latter a contemporary invader. At 7 sites from Maine to Rhode Island, we surveyed crabs for trematode infection prevalence and abundance, and the influence of parasitism on host body condition. We also conducted DNA sequencing using the 18S rRNA barcoding marker to determine species composition, diversity, and gene flow of trematode lineages among the co-occurring hosts. While the native host, *C. irroratus*, and the historical invader, *C. maenas*, exhibited no statistical difference in trematode prevalence, we found that *C. maenas* had a greater abundance of metacercarial cysts than the other 2 hosts, and the contemporary invader, *H. sanguineus*, was rarely infected. Crab condition did not vary with infection abundance, although infected females of all species had higher reproductive investment than other groups. Genetic analyses revealed that the microphallid trematodes consisted of 3 main clades, representing over 50 haplotypes, with evidence of host-switching by native parasites utilizing the non-native hosts. Given the importance of crustaceans to parasite life cycles, the introduction of novel hosts to these systems alters both free-living and host–parasite community interactions and could ultimately affect community structure and function. Future studies should continue to investigate host–parasite diversity and demographics following invasions to better understand impacts on native marine communities.

KEY WORDS: *Cancer irroratus* · *Carcinus maenas* · *Hemigrapsus sanguineus* · Invasion history · Non-native · New England · Species introduction · Trematode

1. INTRODUCTION

During the Anthropocene, human-mediated species introductions have significantly altered ecological relationships, leading to multiple novel interactions and impacts within marine communities, including among hosts and parasites (Carlton & Geller 1993, Molnar et al. 2008, Laverty et al. 2015, Seebens et al. 2016). In recent decades, species introductions have increased globally due to enhanced shipping technologies and transport efficiencies (Ruiz et al. 1997, Seebens et al. 2017). As a result, contemporary host–parasite communities represent species assemblages amalgamated over multiple time periods and from varying source regions (Blakeslee et al. 2013). Historically, marine introductions (1700s–1800s) predominantly occurred via solid ballast (Minchin et al. 2009, Brawley et al. 2009) with multiple host age classes (larvae, juveniles, adults) represented (Blakeslee et al. 2013, Goedknegt et al. 2016). Contemporary ships use water as ballast, and hitchhiking organisms are primarily in larval stages of development (Minchin et al. 2009). This life stage is less likely to be parasitized because of limited opportunity to contract infection and missing or diminutive target tissues (Torchin & Mitchell 2004). Over time, changes to sources and

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propagule pressure have also influenced the diversity and prevalence of parasite fauna infecting non-indigenous host populations (Blakeslee et al. 2013). Indeed, many introduction vectors remain active, enhancing the likelihood that a diverse array of hosts and parasites (and genotypes) will be introduced to the same region over time via these historical and contemporary vectors (Blakeslee et al. 2013).

Following host establishment, a series of possible changes to host–parasite communities could theoretically occur (Thieltges et al. 2009, Goedknegt et al. 2017). One scenario is that an introduced host leaves behind its parasites ('parasite escape'), providing the non-native host with possible fitness benefits and competitive advantages over native competitors ('parasite release') (Torchin et al. 2001, 2002, Ross et al. 2010). For example, in the Northeast USA, 2 non-native, co-occurring crabs, *Carcinus maenas* and *Hemigrapsus sanguineus*, have both escaped castrating parasites found in their native ranges (Blakeslee et al. 2009). This release from castrators has been shown to confer a fitness advantage to *C. maenas*, in its non-native range in northeastern North America (Torchin et al. 2001). A second scenario is that the introduction of novel competent hosts could result in native parasites 'host-switching' to utilize these new hosts (Torchin & Mitchell 2004). Host-switching could then result in a number of changes to parasite prevalence, abundance, and diversity in native and non-native hosts in the introduced region over time (Krakau et al. 2006, Kelly et al. 2009, Johnson & Thieltges 2010, Poulin 2011, Goedknegt et al. 2016, Wolff & Reise 2022). Given the potential for altered host–parasite dynamics, it is critical to track the abundance and diversity of common parasites through time in introduced and native host populations.

While species introductions can be associated with several parasite groups, one taxon, the digenean trematodes (parasitic flatworms), is especially common in both native and non-native marine hosts (Blakeslee et al. 2013). A typical digenean life cycle involves 3 hosts: a first-intermediate mollusk host, a second-intermediate host of myriad invertebrate and vertebrate taxa, and a vertebrate definitive host (Rohde 2005, Galaktionov et al. 2012). Due to widespread distributions in native and non-native communities, trematodes have been investigated in multiple studies examining the influence of species introductions on parasite communities (e.g. Miura et al. 2006, Reisinger & Lodge 2016). For example, relevant work by Blakeslee et al. (2020a) examined the effect of host introduction on trematode communities (family Microphallidae) in 2 Newfoundland (Canada) bays: one invaded by the European green crab *C. maenas* and one not. In both bays, the native rock crab *Cancer irroratus* and 2 native species of *Littorina* snails were surveyed for trematode parasites in addition to *C. maenas*. The study found native trematode parasites using the invasive *C. maenas* crab as a competent host, and the addition of this new host diluted trematode abundance in native *C. irroratus* crabs in the invaded bay. Moreover, past work on *C. maenas* identified host–parasite coevolutionary history to play a strong role on parasite prevalence and abundance in terms of differential susceptibility to trematode infection in native and non-native ranges (Blakeslee et al. 2020b). Thus, contact timing with a parasite is an integral factor affecting parasite prevalence and abundance in host–parasite communities, and is an important aspect to consider when examining historical versus contemporary invasions of hosts to novel regions.

In this study, we furthered examinations of trematode diversity in native *C. irroratus* and non-native *C. maenas* by exploring questions of coevolutionary history and invasion timing in the Northeast USA, where the crabs have overlapping distributions. In our expanded investigation, we also included a third host, the Asian shore crab *H. sanguineus*, which invaded the Northwest Atlantic much more recently (i.e. it is a contemporary invader). While this species has also escaped parasites (Blakeslee et al. 2009), recent evidence suggests it may be infected by more parasites in USA populations than in original surveys, including digenean trematodes (Kroft & Blakeslee 2016). Past work has also examined the effect that trematode infection may have on host physiology, particularly using body condition indices in both *C. maenas* (Blakeslee et al. 2015) and *H. sanguineus* (Kroft & Blakeslee 2016). These studies found mixed results of the role of parasites on body condition in their invaded populations, but nothing has yet been examined in *C. irroratus*, nor comparatively across all 3 host crabs. Therefore, the objectives of our study were to determine the overlap and extent of microphallid trematode infection in the 3 crab hosts, how coevolutionary history may impact parasite diversity in each, and whether infection may differentially affect host condition (reproduction and energy storage) in the crabs' co-occurring populations. We conducted 2 parasite surveys in 2017 in the Northeast USA and examined trematode prevalence, abundance, and host condition in the 3 crab species: *C. maenas* (historical invader), *H. sanguineus* (contemporary invader), and *C. irroratus* (native, with long-term coevolutionary history). We also conducted a

genetic survey from multiple Northwest Atlantic sites and some Northeast Atlantic sites in the native range of *C. maenas* to help resolve the identities, overlap, and gene flow of microphallid trematodes infecting the 3 crab species.

2. MATERIALS AND METHODS

2.1. Study system

Carcinus maenas was first documented in the Northwest Atlantic in 1817, having been introduced via solid ballast or ship fouling from Europe (Carlton & Cohen 2003). By the 1980s, the crab was found from New Jersey, USA, to Halifax, Nova Scotia, Canada (Roman 2006). A second major introduction from northern Europe to eastern Nova Scotia via ballast water occurred in the 1990s, resulting in new genotypes spreading throughout Canada and into the USA (Roman 2006, Lehnert et al. 2018). Secondary spread from the Halifax region resulted in the introduction of *C. maenas* to Newfoundland in the early 2000s (Blakeslee et al. 2010), where the crab has continued to expand its range. US populations of *C. maenas* primarily derive from the historical introduction in the 1800s, although some mixture of the historical and contemporary introductions now occurs in northeastern USA populations (Lehnert et al. 2018).

Hemigrapsus sanguineus was first documented at the mouth of Delaware Bay in 1988 and has since spread north and south along the US Atlantic coast, presently ranging from central-northeastern Maine to Beaufort, North Carolina (McDermott 1998, Epifanio 2013, Lord & Williams 2017). The crab invaded the US from Asia, likely originating from Japan, and genetic analyses suggests there may have been multiple introductions (Blakeslee et al. 2017, Lord & Williams 2017). The introduction vector was likely ballast water (Epifanio 2013, Blakeslee et al. 2017).

Past work examining parasite communities in *C. maenas* versus *H. sanguineus* (Torchin et al. 2001, Blakeslee et al. 2009) have found greater parasite loads in the historical (*C. maenas*) versus contemporary (*H. sanguineus*) invader that appear to be associated with several factors including time since introduction, source region, and introduction vectors (Blakeslee et al. 2009). A more recent examination of parasite diversity in *H. sanguineus* compared to native panopeid mud crabs (*Eurypanopeus depressus* and *Panopeus herbstii*) found that parasite diversity in *H. sanguineus* has increased with time but still remains significantly lower compared to its native region (Kroft & Blakeslee 2016). Further, the recent investigation (Blakeslee et al. 2020a) of *C. maenas* (historical invader) versus *Cancer irroratus* (native) microphallid parasites in Newfoundland found evidence of host-switching in native trematodes to utilize the non-native crab host. However, to date, no study has performed a comparative investigation in all 3 host crabs in their overlapping ranges in the Northeast USA.

2.2. Host and parasite sampling

Two field collections were conducted in 2017 (May and August) with sites from Maine to Rhode Island, USA (Table 1, Fig. 1). These surveys obtained microphallid trematode parasite and host data from *C. irroratus* (native), *C. maenas* (historical invader), and *H. sanguineus* (contemporary invader). We collected 3–15 individuals per species per site at 6 sites during the first survey in May 2017, and 4 sites during the second survey in August 2017 (Fig. 1). The second survey was to capture potential seasonal differences

Table 1. Survey information, including site names, latitudes and longitudes, and numbers of infected individuals/total number sampled per crab species, demonstrating proportion infected. '–': No crabs detected at the site, na: site was not visited during the sampling period; CI: *Cancer irroratus*, CM: *Carcinus maenas*, HS: *Hemigrapsus sanguineus*; ME: Maine, NH: New Hampshire; MA: Massachusetts; RI: Rhode Island

Site	Latitude (N)	Longitude (W)	Survey 1 (June 2017)			Survey 2 (August 2017)		
			CI	CM	HS	CI	CM	HS
Camden, ME	44° 12′ 32.32″	69° 3′ 28.44″	–	15/15 = 1.00	–	–	6/6 = 1.00	–
Orr Island, ME	43° 47′ 22.54″	69° 57′ 36.44″	4/5 = 0.80	15/15 = 1.00	0/15 = 0.00	na	na	na
Rye, NH	43° 0′ 2.02″	70° 44′ 39.38″	–	12/15 = 0.80	0/15 = 0.00	0/3 = 0.00	11/15 = 0.73	0/13 = 0.00
Gloucester, MA	42° 36′ 28.91″	70° 40′ 34.81″	–	9/14 = 0.64	1/15 = 0.07	na	na	na
Scituate, MA	42° 12′ 14.17″	70° 43′ 0.24″	5/6 = 0.83	13/15 = 0.87	4/15 = 0.27	2/7 = 0.29	–	0/14 = 0.00
Providence, RI	41° 49′ 3.71″	71° 23′ 31.75″	na	na	na	–	0/10 = 0.00	0/15 = 0.00
Weekapaug Point, RI	41° 19′ 32.52″	71° 41′ 10.03″	–	–	0/15 = 0.00	na	na	na
Total sampled			11	74	75	10	31	42

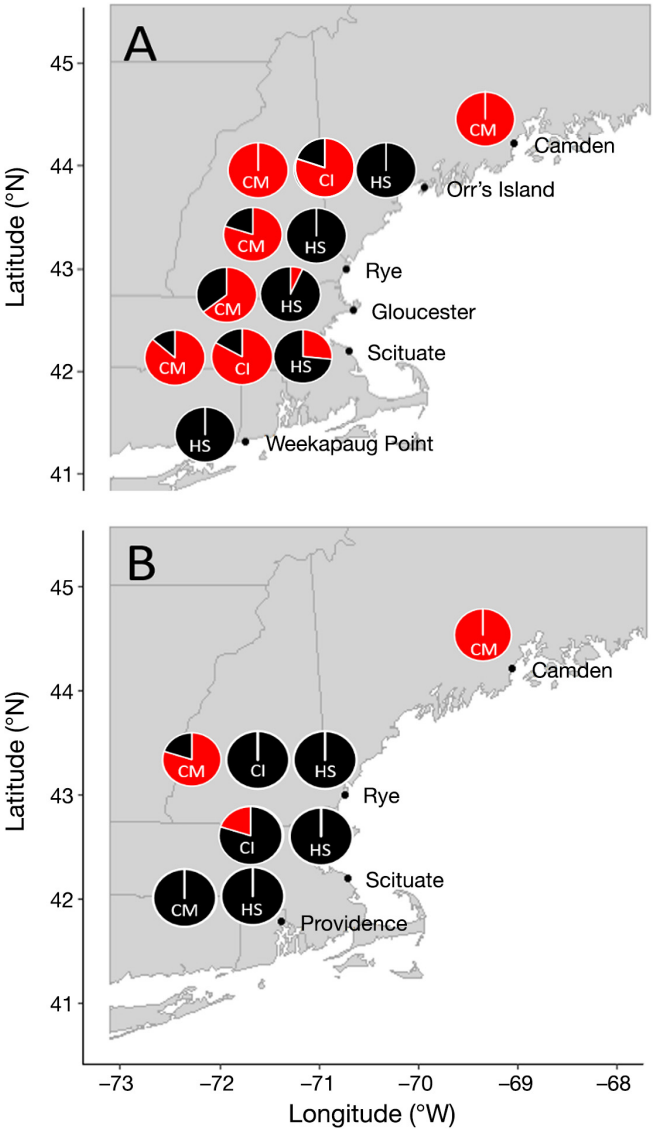

Fig. 1. Prevalence of infection in 3 crab hosts in (A) May and (B) August 2017. Red indicates infection while black indicates lack of infection. CI: *Cancer irroratus*; CM: *Carcinus maenas*; HS: *Hemigrapsus sanguineus*. See Table 1 for sample sizes of each crab per survey month

in microphallid prevalence and intensity from spring to summer. While we attempted to sample 15 individuals of all 3 crabs at each site, similar to past efforts (e.g. Blakeslee et al. 2020a), we were sometimes unable to find all crab species and/or 15 individuals (Table 1; Table S1 in the Supplement at www.int-res.com/articles/suppl/m697p067_supp.pdf). This may have affected our ability to detect some patterns in our dataset, particularly related to analyses that examined infection status. For *C. irroratus* and *C. maenas*, we used baited collapsible crab traps attached to the shore or a dock for 1 tidal cycle. For *H. sanguineus*, we performed hand collections at low tide in the mid- to upper intertidal zone by flipping intertidal rocks and cobble. We transported the crabs back to the lab on ice where they were then frozen in a −20°C freezer until dissection.

2.3. Crab dissections

Prior to dissection, each crab was measured using digital calipers to obtain the maximum carapace width (mm). Dissection protocols for parasite analyses followed previous studies (Blakeslee et al. 2009, 2015, 2020a). Using a sterilized razor blade, the upper carapace was separated from the lower carapace to expose internal tissues. Eight tissue snips, or the amount of tissue that filled a 22 × 22 mm coverslip, were then removed from the crab: 6 from the hepatopancreas (energy storage tissue), 1 from the gonad, and 1 from the thoracic ganglia (nerve center). Each slide containing tissue snips was systematically scanned for trematode cysts using a Zeiss compound microscope at 4× with 10× oculars. When microphallid trematode cysts were found, they were enumerated using a hand-held tally counter. Cysts were identified based on morphological features (James 1968, Blakeslee et al. 2009, 2015, 2020a) and also confirmed with genetic analyses. For the genetic work, cysts were moved into 1.5 ml tubes and stored in a −20°C freezer until processing. Following dissections, all tissues from each crab collected during the May 2017 survey, along with the bodies of these crabs, were moved to foil containers for drying in an oven to analyze for body condition (hepatosomatic and gonadosomatic indices, HSI and GSI, respectively).

2.4. Hepatosomatic and gonadosomatic indices

For crabs collected during the May 2017 survey, we examined body condition indices by crab species, infection status, and infection intensity (i.e. for infected crabs only). The HSI and GSI were calculated as the ratio of dried hepatopancreas or gonad to the overall dried body weight of a crab (Kennish 1997) after subtracting the expected contribution of metacercarial cysts to the tissue mass. Refer to Blakeslee et al. (2015) for detailed methodology regarding the calculation of metacercarial cyst mass. We corrected for any missing limbs using regressions of limb weights and carapace width from previous published data for each crab species: *C. maenas* (Blakeslee et al. 2015),

C. irroratus (Blakeslee et al. 2020a), and *H. sanguineus* (Kroft & Blakeslee 2016).

2.5. Statistical analyses for prevalence, abundance, and body condition

Microphallid trematode prevalence per site was calculated as the number of infected crabs divided by the total number of crabs examined. Microphallid trematode abundance per crab was defined as the total cyst count in each crab, and for some crabs this amounted to a zero value (i.e. the abundance measure includes uninfected and infected crabs; Bush et al. 1997). Analyses of cyst abundance focused on the hepatopancreas because trematode cysts primarily concentrate in these tissues (Torchin et al. 2002, Blakeslee et al. 2009, 2020a). Because the 3 hosts vary in body size and total volume of tissue, we standardized abundance measures by estimating the cysts per gram of hepatopancreas tissue. This value was estimated from an average number of cysts per hepatopancreas snip, which was then multiplied by the differential for a gram of hepatopancreas tissue using a standardized tissue weight per snip (0.116 g) calculated from a number of prior data points (n = 75) (Blakeslee et al. 2015, 2020a, this study).

Crab infection prevalence and abundance were analyzed using generalized linear models (GLMs), with sampling period (May or August), species, sex, and size as fixed effects. In preliminary runs, no interactions were significant. Prevalence GLMs had a binomial distribution with a probit link function, and abundance GLMs had a negative binomial probability distribution with a log link function.

HSI was analyzed using a linear model with a normal distribution (identity link), with species, size, sex, cyst abundance, and the interaction of size and species as fixed effects (no other significant interactions were detected during preliminary runs). GSI was analyzed using a GLM with a gamma distribution (log link), with species, size, sex, cyst abundance, and the interaction of species and sex as fixed effects. Statistical analyses were performed using SPSS v26.

2.6. Trematode identities and genetic analyses

2.6.1. Samples included in genetic analyses

To determine trematode identities in the 3 host crabs, we included a subset of specimens from our surveys for DNA sequencing and additional sites in New Brunswick and Nova Scotia from other unpublished work, providing a dataset of new sequences that we amalgamated with previously published and unpublished microphallid sequences from these crabs (Blakeslee et al. 2020a). Previously published sequences (Blakeslee et al. 2020a) included additional samples from Newfoundland and Europe. We also included sequences for 2 microphallid cysts and 5 excysted microphallid individuals from *H. sanguineus* in Long Island, USA, that were part of the study by Kroft & Blakeslee (2016) but had not previously been sequenced. Altogether, our genetic dataset of new and prior microphallid sequences includes a total of 138 sequences from *C. maenas*, 37 from *C. irroratus*, and 9 from *H. sanguineus* (GenBank accession numbers: MT025331–MT025339, MT025341–MT025347, MT025349–MT025355, and OP125517–OP125533). We were unable to collect as many *C. irroratus* crabs as the other 2 species (Table 1), especially where they overlapped, which accounts for the discrepancy in sequencing numbers among the crabs. For *C. maenas*, additional sequences also come from Canadian and European sites (Table S1). For *H. sanguineus*, we sampled 117 individuals, but very few were infected (Table 1).

To form a more comprehensive phylogenetic tree of microphallid sequences, we also included overlapping sequences from other North American microphallids, including: (1) *Microphallus turgidus* (accession no. EU825773; Cho 2012), which infects multiple crustaceans in the Northwest Atlantic (Pung et al. 2006); (2) *M. primas* (accession no. AJ287541; Littlewood & Olson 2001), which infects *C. maenas* in the Northeast Atlantic (James 1968); (3) *Gynaecotyla adunca*, which infects the mud snail *Ilyanassa obsoleta* as the first-intermediate host and multiple crustaceans as second-intermediate hosts (Hunter & Vernberg 1953); and (4) *Maritrema arenaria*, which was sampled from an upstream snail host, *Littorina saxatilis*, and infects barnacles as second-intermediate hosts (Blakeslee et al. 2020a). This latter species was used as the outgroup to root our phylogenetic tree. Finally, we included microphallid sequences previously collected from an upstream snail host (*L. saxatilis*) in the Northeast Atlantic (Blakeslee et al. 2020a) for additional understanding of microphallid lineages across the North Atlantic.

2.6.2. DNA extraction and sequencing methodologies

DNA was extracted using a standard CTAB/chloroform/ethanol precipitation (France et al. 1996, Blakeslee et al. 2020a). DNA concentration and puri-

ties were determined using a Nanodrop 8000 (Thermo Scientific). A 468 bp fragment of the 18S rRNA gene was then amplified using primers designed from the *M. turgidus* sequence described above. PCR was performed with the profile from Blakeslee et al. (2020a). PCR amplicons were purified with ExoSAP-IT™ (ThermoFisher) and Sanger sequenced at Psomagen (Rockville, MD, USA). Sequences were manually cleaned, inspected for ambiguities, and aligned to the *M. turgidus* reference sequence using Geneious 10.1.2 (Biomatters). Resulting sequences were collapsed into haplotypes with TCS 1.21. Phylogenetic relationships were determined using Bayesian reconstructions (burn-in: 100 000; total chain length: 1 000 000) with MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001) in Geneious 10.1.2. Microphallid lineages were defined as sequences possessing 97–100% similarity and grouping into clades or subclades. We used BLAST to examine sequences deposited into GenBank that were top hits for our representative sequences for each clade or subclade (Table S2). Given the limited sequencing of North American microphallids, many of these matched to sequences recently deposited by Blakeslee et al. (2020a).

3. RESULTS

3.1. Prevalence and abundance of host crabs in US surveys

Prevalence and abundance of microphallid trematodes infecting the 3 crabs were analyzed to determine influential factors driving these response variables. Crab species had a significant influence on prevalence ($F = 61.880$; $p < 0.001$), with *Hemigrapsus sanguineus* having significantly lower infection prevalence ($p < 0.05$) than the other 2 crab species, but there was no significant difference between *Carcinus maenas* and *Cancer irroratus*. Sampling period was also significant ($F = 12.685$; $p < 0.001$), with the May sampling period having significantly higher prevalence than the August sampling period. There was no influence of sex ($F = 0.905$; $p = 0.439$) on prevalence. Size was marginally significant ($F = 3.57$; $p = 0.060$).

In abundance analyses, crab species had a significant effect on microphallid cyst abundance ($F = 13.36$; $p < 0.001$), with *C. maenas* having significantly greater abundance of trematode cysts ($p < 0.05$) than the other 2 crab species; there was no difference between *C. irroratus* and *H. sanguineus* (Fig. 2). Sampling period ($F = 0.658$, $p = 0.419$), sex ($F = 1.262$; $p = 0.287$), and size ($F = 1.80$; $p = 0.181$) were not significant factors.

3.2. Body condition indices

We found a significant influence of species, size, sex, and the interaction of species and size, but no influence of cyst abundance on HSI (Table S3). *H. sanguineus* had the highest HSI of the 3 crabs (Fig. 3A), and collectively across all crabs, males had higher HSI than females (Fig. 3B). Size showed a significant negative relationship with HSI for *C. maenas* (R^2 adj = 0.152; $p = 0.008$; Fig. 3C), but the relationships were not significant for *C. irroratus* or *H. sanguineus*. All 3 crabs demonstrated different size ranges, with *H. sanguineus* having the smallest size range, *C. maenas* intermediate, and *C. irroratus* the largest (Fig. 3C). For GSI, there was a significant influence of species, sex, and the interaction between sex and species, but no influence of cyst abundance or size (Table S4). Females had significantly ($p < 0.05$) higher GSI than males, and *C. maenas* had higher GSI than the other 2 species. Female *C. maenas* had a higher GSI than the other species and sex combinations (Fig. 3D).

In a separate analysis of infection status rather than infection intensity, there was no significant effect of infection status on HSI (Table S5). For GSI, infection status on its own was not a significant factor; however, the interaction of infection status and sex was significant, with infected females demonstrating the highest GSI values (Table S6, Fig. S1).

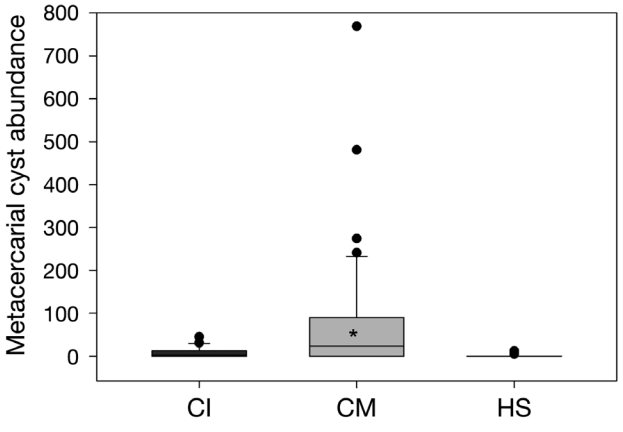

Fig. 2. Metacercarial cyst abundance (cysts per gram hepatopancreas) for the 3 host crab species. *Significant ($p < 0.05$). Box plots depict the 10th, 25th, 75th, and 90th percentiles as boxes and error bars, with lines representing the median. Individual dots are outliers. CI: *Cancer irroratus*; CM: *Carcinus maenas*; HS: *Hemigrapsus sanguineus*

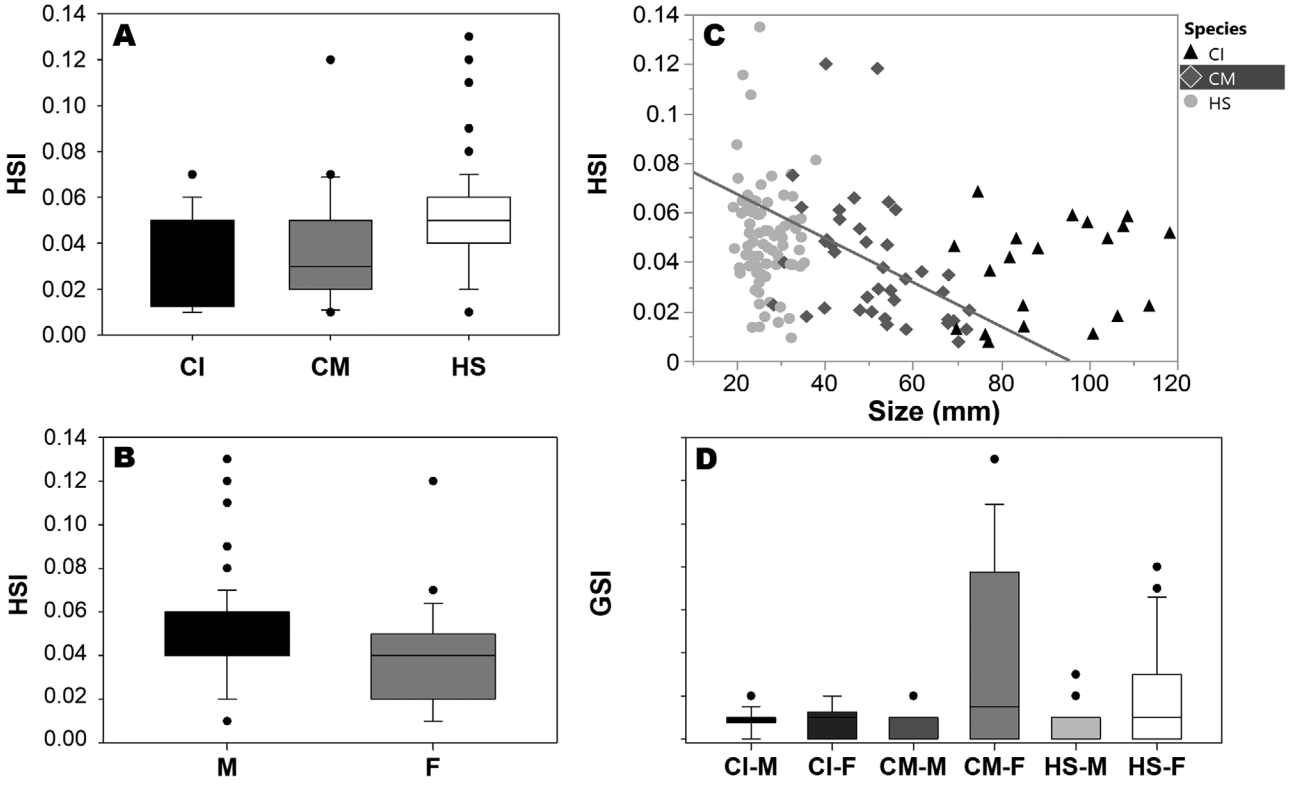

Fig. 3. Crab hepatosomatic index (HSI) and gonadosomatic index (GSI) analyses demonstrating significant driving factors of (A) species for HSI (CI: *Cancer irroratus*; CM: *Carcinus maenas*; HS: *Hemigrapsus sanguineus*), (B) sex for HSI, (C) size for HSI, and (D) species and sex for GSI. Infection status was not significant for any species; thus these data represent pooled infected and uninfected individuals per species. In (C), individual species are designated by different icons (see key). CM is highlighted in the key because there is a significant negative relationship (R^2 adj = 0.152; p = 0.008) between HSI and size; there was no significant relationship for the other 2 species

3.3. Trematode identities and biogeography

Genetic analyses of microphallid cysts from the 3 host crabs uncovered a total of 33 haplotypes in North America and Europe (Table S1, Fig. 4). Based on genetic similarities, these haplotypes were distributed among 3 major clades (A,B,C) and 4 subclades within A (A.1, A.2, A.3, A.4). Using sequence data from previously identified microphallids in North America and Europe, it appears that the microphallids in our study are represented by at least 4 trematode species (Table S2), including *Microphallus similis* (lineage B), *M. primas* (lineage C), *M. turgidus* (possibly lineage A.2), and *Gynaecotyla adunca* (A.4), with 2 lineages (A.1, A.3) unidentified. Further, haplotypes were detected in Clade A that placed outside these 4 'A' subclades and could represent additional cryptic taxa (Table S1, Fig. 4).

Both Clades A and B were found in North America. Clade C was only found in *C. maenas* in Europe and matched with the trematode species *M. primas*. *M. similis* was detected in North America and Europe, but the other 2 microphallid species (*M. turgidus* and *G. adunca*) were only found in North America. Three haplotypes (2, 4, 5) from clades A and B were identified in both *C. maenas* and *C. irroratus*, and 1 haplotype (5) was cosmopolitan across species and geographic region. Interestingly, although this latter haplotype was the most frequent haplotype (representing 53% of all detected haplotypes), it was not found in *H. sanguineus*. In fact, all 9 of the microphallid individuals found infecting *H. sanguineus* and sequenced for this work were from Clade A, lineages A.2–A.4. Moreover, several of the Clade A sequences were only found in *C. irroratus*, the native North American crab (Fig. 3).

4. DISCUSSION

Our study examined the effect of species introductions on North American trematode parasite communities along a gradient of host coevolutionary history, including contemporary (~30 yr) and historical

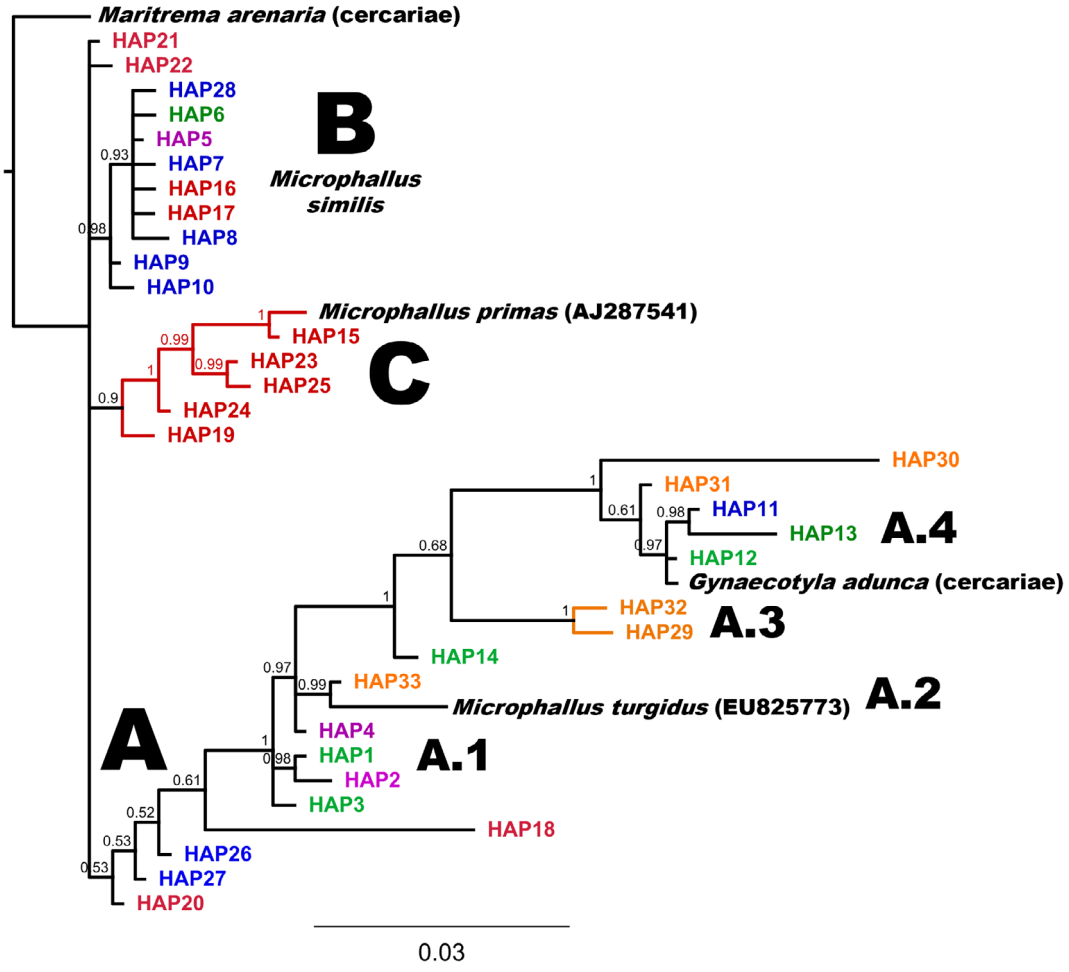

Fig. 4. Bayesian phylogenetic tree of the 18S rRNA barcoding gene (468 bp fragment) of microphallid trematodes in our study. Large letters represent the major clades in the tree, and A.1–A.4 represent subclades based on genetic similarities of 98–100%. Colors represent host crab species and bioregions: green = *Cancer irroratus*, North America; blue = *Carcinus maenas*, North America; purple = *C. maenas* + *C. irroratus*, North America; red = *C. maenas*, Europe; orange = *Hemigrapsus sanguineus*, North America. There were no combinations of *H. sanguineus*-hosted trematodes shared with the other 2 crab species. The outgroup and root for the tree is *Maritrema arenaria*. Numbers at nodes represent posterior probabilities

(~200 yr) invaders and a coevolved native species. We found that trematode prevalence depended on host species, with the most recent inhabitant (*Hemigrapsus sanguineus*) having the lowest prevalence among the 3 crab species, while the historical invader (*Carcinus maenas*) and the native crab (*Cancer irroratus*) were not significantly different. Trematode identities were much more complex than originally expected, with signatures of 3 distinct clades and additional subclades. Crab body condition was not significantly influenced by trematode infection intensity in our study, but we did find evidence of species and sex effects on energy storage, as well as an effect of infection status on GSI in female crabs. Here, we discuss these results in greater detail and highlight their implications to our understanding of the impact of species introductions on host–parasite communities associated with biological invasions.

4.1. Prevalence of trematode infection and metacercarial abundance and intensity

Past work has shown that coevolutionary history and time since introduction can affect host infection prevalence and abundance (e.g. Guégan & Kennedy 1993, Torchin et al. 2001, 2002, Kołodziej-Sobocińska et al. 2018). Similarly, we found that trematode infection prevalence and metacercarial abundance differed among the 3 hosts, which largely corresponded with coevolutionary history. Specifically, the contemporary invader *H. sanguineus* showed significantly

lower trematode prevalence compared to the historical invader *C. maenas*. In addition, both native *C. irroratus* and the historical invader *C. maenas* hosted a significantly greater abundance of metacercarial cysts than did the contemporary invader *H. sanguineus*. Indeed, in separate experimental studies in both *C. maenas* and *H. sanguineus*, coevolutionary history was shown to influence prevalence and metacercarial abundance when native and non-native crabs, respectively, were exposed to native and non-native microphallid cercariae (Keogh et al. 2017, Blakeslee et al. 2020b).

In our system, the contemporary invader *H. sanguineus* has only recently (2014) been detected harboring metacercarial cysts in North America (reported by Kroft & Blakeslee 2016); a prior study from 2007 found no trematode infections (Blakeslee et al. 2009). In our study in 2017, we found additional records of trematode metacercariae in *H. sanguineus*, and these appear to be from multiple trematode lineages within Clade A (Fig. 3). Although these trematode infections continue to be relatively rare in the contemporary invader, they do suggest that the crab now serves as a competent host to some presumably native microphallid species in the non-native range of the crab. Yet, when compared to the historical invader *C. maenas* and the native species *C. irroratus*, prevalence of microphallid infection was significantly lower in *H. sanguineus*, indicating that the contemporary invader is still experiencing greater levels of parasite escape than the other 2 crab species.

Counter to expectations, we did not observe higher infection prevalence in native *C. irroratus* versus the historical invader *C. maenas*. Interestingly, where *C. irroratus* and *C. maenas* co-occurred, *C. maenas* demonstrated higher infection prevalence and abundance than *C. irroratus*. Potential explanations could include the longer coevolutionary history of *C. irroratus* with North American trematodes, which may have resulted in a predictably greater resistance of infection for the native versus non-native crab (e.g. similar to a study of native versus non-native *C. maenas* populations; Blakeslee et al. 2020b). Another possibility is that a greater diversity of trematode lineages parasitizes *C. maenas* in the region, including lineages co-introduced with *C. maenas*. A third explanation could be a sampling issue, in that fewer *C. irroratus* crabs were found at our sites than *C. maenas*, possibly due to the latter outcompeting the former (Dumas & Witman 1993, Matheson & Gagnon 2012). Alternatively, our sampling method of targeting the shallow subtidal zone may have affected our ability to capture *C. irroratus*, which could be inhabiting deeper subtidal waters in the region. Indeed, the location of a host within the intertidal/subtidal zone may influence its contact with microphallid trematodes, which are emitted as free-swimming cercariae from infected first-intermediate snail hosts and seek out a second-intermediate crustacean host (Blakeslee et al. 2020a). As the first-intermediate snails in our system inhabit the intertidal zone, their proximity to second-intermediate hosts could play a role in infection burden in these downstream crab hosts. However, *H. sanguineus*, which is more closely tied to the intertidal zone than *C. maenas* or *C. irroratus*, demonstrated the lowest abundance of trematode metacercarial cysts in our surveys (Fig. 2). Thus, proximity to cercariae does not appear to explain the more limited infection of *H. sanguineus* compared to the other 2 crab species. Instead, it may suggest that *H. sanguineus* is not, at present, a competent host for the most abundant microphallid species (*M. similis*) found at our sites and which predominantly infected *C. maneas* and *C. irroratus* in the region (Fig. 4). This trematode species uses rocky intertidal *Littorina* spp. snails as first-intermediate hosts (James 1968) and was present at the sites where *H. sanguineus* was sampled.

Temporal differences in infection prevalence were also detected in our study: infection prevalence and abundance dropped from May to August in all 3 crab species. Generally speaking, we captured fewer individuals during our August sampling, unexpectedly failing to sample even a single *C. maenas* individual at 1 of our sites, Scituate, Massachusetts, where this species had been sampled previously. This drop in *C. maenas* collected during the second survey could possibly be explained by reproduction behavior, with crabs forming mating pairs in the subtidal (Berrill 1982) outside of where we sampled. While this mating ritual typically occurs from late August through mid-October, warming temperatures may be shifting the mating season earlier, as climate change has been linked to other temperature-dependent animal behaviors (Van Buskirk et al. 2009, Jones et al. 2010, Jensen et al. 2018). Moreover, infection prevalence may also decline in crabs while they are exhibiting these behaviors, as it may be more difficult for the free-swimming stage of the trematode (cercariae) to reach crabs in the deeper subtidal (Blakeslee et al. 2020a).

4.2. Identity of trematode parasites with biogeography

Host-switching is a phenomenon whereby parasites may utilize novel competent hosts (like invaders) in a

system, and this could influence host–parasite interactions within these communities (Goedknegt et al. 2016). The likelihood of host-switching may depend on the generality of the life cycle at the stage in question and, once again, the time since host introduction. In our system, we clearly identified evidence of host-switching in the Northeast USA in both invaders. First, microphallid subclades A.2 and A.4, which were found in infected *C. maenas* and *H. sanguineus* (Fig. 4), appear to represent *Microphallus turgidus* and *Gynaecotyla adunca*, respectively, and are native North American species that infect first-intermediate hydrobiid and mud snails (Heard & Overstreet 1983, Pung et al. 2009, West et al. 2014). These trematodes were present in greater diversity and abundance in *C. irroratus* than in the other 2 hosts, suggesting that host-switching may have occurred with the addition of *C. maenas* and *H. sanguineus* as novel hosts. Second, microphallid Clade B was identified as *M. similis*, a cosmopolitan trematode species found in *C. maenas* on both sides of the Atlantic and in *C. irroratus* in North America. Past studies have also detected *M. similis* on both sides of the Atlantic in upstream snail hosts (James 1968, Stunkard 1983) and in *C. maenas* in both regions (James 1968, Blakeslee et al. 2020a). The definitive hosts are a wide variety of shorebirds, which can sometimes have impressive ranges in the North Atlantic, possibly driving the widespread distribution of this lineage (Good 1998). It is also possible that the multiple introductions of *C. maenas* could have transported some *M. similis* genotypes to North America; however, it would require more genetic markers as well as parasites from snail and crab hosts on both sides of the Atlantic to help resolve this question.

It is not uncommon for introduced species to acquire parasites in their novel environments (Goedknegt et al. 2016); however, detecting the level of haplotype diversity of microphallids in *H. sanguineus* in particular was unexpected. Metacercarial cysts have only recently been detected in *H. sanguineus* (Kroft & Blakeslee 2016); thus to observe 5 unique trematode haplotypes among 3 subclades in this host species was surprising. Overall, we detected about twice as many haplotypes for *C. maenas* (11) compared to *H. sanguineus* (5). This may reflect the longer history in North America for *C. maenas*, as similarly observed in other species with longer colonization times (Guégan & Kennedy 1993, Torchin et al. 2001).

Future work can elucidate whether outcomes like parasite dilution (whereby the addition of new competent hosts reduces the parasite load in native hosts), parasite spillback (i.e. the amplification of parasite transmission to native hosts due to the introduction of new competent hosts), or parasite spillover (parasites introduced with a novel host infect native hosts) are occurring in these communities, as investigated in a prior study comparing *C. maenas* and *C. irroratus* in Newfoundland, which detected signatures of parasite dilution in the native host (Blakeslee et al. 2020a). It would be interesting to determine if dilution effects are also apparent in other North American intertidal/shallow subtidal communities, particularly given that these communities have 2 non-native intermediate hosts (*C. maenas* and *H. sanguineus*). Furthermore, while several studies have found evidence of parasite escape and release in *C. maenas* in its non-native North American range, these studies have primarily focused on metazoan macroparasites (e.g. Torchin et al. 2002, Blakeslee et al. 2009, 2013); however, work by Bojko et al. (2018) in Canada and the UK found that when microparasites were also included in parasite diversity analyses, *C. maenas* was host to numerous taxa of parasites and marine symbionts in non-native Canadian populations. Thus, microparasites may be more readily acquired in introduced regions, or more likely to be transported with introduced hosts, than macroparasites, especially those macroparasites with multi-host life cycles. We did not examine microparasites in this study and so cannot assess the potential for host-switching of these parasite groups across the co-occurring crabs we investigated here. Future work could help resolve this question.

4.3. Energy storage and reproductive investment

We used body condition indices as a tool to look for demographic differences in our 3 crab hosts and whether trematode infection intensity was an influential factor (Fig. 3). Specifically, the HSI provides understanding of an organism's investment in energy storage relative to body weight, while the GSI examines reproductive investment. Neither infection intensity nor infection status was a significant factor explaining energy storage in our crab species; however, demographic predictors like species, sex, and size were. Additional sampling of infected individuals may be needed to detect an effect of infection status on these energy storage indices. Energy storage was relatively similar between *C. maenas* and *C. irroratus* but was significantly greater in *H. sanguineus*. Past studies have similarly shown *H. sanguineus* to invest more in energy storage than co-occurring crabs, including panopeid mud crabs (Freeman et al. 2016)

and *C. maenas* (Jungblut et al. 2018). Jungblut et al. (2018) argued that these features may contribute to the success of *H. sanguineus* in invasive European populations. Moreover, Griffen et al. (2012) revealed diet plasticity in introduced versus native ranges of *H. sanguineus*, which may also contribute to its success in invasive populations.

Crab sex was also a contributing factor to HSI, with males allocating slightly more investment towards energy storage than females. Given that our GSI analyses demonstrated the opposite result (females having higher GSI, particularly for *C. maenas* and *H. sanguineus*), this may reflect opposite strategies where females must invest more towards reproduction than males, thereby having to invest less in energy storage (Kyomo 1988, Hamid et al. 2016), i.e. a tradeoff in investment of energy storage versus reproduction (Griffen et al. 2011). However, seasonal differences can also affect the relative contributions to energy storage and reproduction among males and females, particularly during times of mating or egg production (Dima et al. 2009). Our HSI/GSI analyses were based on a single time point (May) and so do not reflect changes that could occur in these indices over the year. GSI analyses further indicated an interaction between species and sex, with female *C. maenas* showing the largest investment in reproduction compared to the other combinations of species and sexes. Once again, it may be that the investments in reproduction of the 3 species occur at different times of the year, and our sampling in May captured a greater investment by female *C. maenas* at this time point compared to the others. It may also reflect differences among the crabs in other ecological factors, including diet, competition, and predation risk (Griffen et al. 2011). Interestingly, although neither infection intensity nor infection status alone were significant predictors of GSI, the interaction of infection status and sex was significant, with infected females demonstrating greater GSI than the other combinations. While trematodes encysting in the hepatopancreas have not been shown to castrate hosts or seriously affect reproduction, their presence in female crabs in our study may have had some influence on increased reproductive investment as a response to infection, which has previously been observed in male crabs (Zetlmeisl et al. 2011).

Finally, size was a significant factor of HSI in *C. maenas*, which showed a weak but significant decline in HSI with increased crab size. This suggests that younger, smaller *C. maenas* crabs are investing more in energy storage than older, larger crabs. As the energy required for molting is stored in the hepatopancreas (Kennish 1997) and younger crabs have to molt more frequently, a greater investment in energy storage may be more likely in smaller, younger crabs than larger, older crabs that are approaching their terminal molt (Griffen et al. 2011).

4.4. Conclusions and implications

In recent years, biological invasions have become recognized as a major contributor to changes in community structure and function. However, much of this recognition has focused on free-living communities, with much less attention provided to host–parasite communities (Blackburn & Ewen 2017). Given the strong behavioral, physiological, and fitness effects that parasites often exert on their hosts, this limited understanding suggests we are missing a substantial piece of the puzzle in terms of tracking community changes post-invasion (Marcogliese & Cone 1997). Moreover, because species invasions have been continually occurring over the past few centuries, invaded communities represent an amalgamation of recent and historical introduction events of hosts and parasites, with their impact and spread changing through time (Blakeslee et al. 2013). This further complicates our global understanding of parasite phylogenies, which remain under-resolved compared to free-living taxa. For example, like others, we detected several cryptic or unidentified parasite lineages (Huspeni 2000, Miura et al. 2005, 2006, Cai et al. 2020, Giulietti et al. 2020). These findings demonstrate how important it is to reevaluate our assumptions of species diversity, especially as it applies to introduced species and their interactions, since some introductions (particularly parasites) may be cryptic and hidden from view. Further studies can elucidate the taxonomies and biogeographies of parasite lineages, like the ones here, and their particular influences on marine hosts and the broader community. Given the importance of crustaceans as hosts to multiple parasite species, species invasions that alter free-living and host–parasite community interactions can have major consequences on community structure and function.

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LITERATURE CITED

Berrill M (1982) The life cycle of the green crab *Carcinus maenas* at the northern end of its range. J Crustac Biol 2: 31–39

Blackburn TM, Ewen JG (2017) Parasites as drivers and passengers of human-mediated biological invasions. EcoHealth 14:61–73

Blakeslee AMH, Keogh CL, Byers JE, Kuris AM, Lafferty KD, Torchin ME (2009) Differential escape from parasites by two competing introduced crabs. Mar Ecol Prog Ser 393:83–96

Blakeslee AMH, McKenzie CH, Darling JA, Byers JE, Pringle JM, Roman J (2010) A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of an invasive marine crab to Newfoundland. Divers Distrib 16:879–891

Blakeslee AMH, Fowler AE, Keogh CL (2013) Marine invasions and parasite escape: updates and new perspectives. Adv Mar Biol 66:87–169

Blakeslee AMH, Keogh CL, Fowler AE, Griffen BD (2015) Assessing the effects of trematode infection on invasive green crabs in Eastern North America. PLOS ONE 10: e0128674

Blakeslee AMH, Kamakura Y, Onufrey J, Makino W and others (2017) Reconstructing the invasion history of the Asian shorecrab, *Hemigrapsus sanguineus* (De Haan 1835) in the Western Atlantic. Mar Biol 164:47

Blakeslee AMH, Barnard RB, Matheson K, McKenzie CH (2020a) Host-switching among crabs: species introduction results in a new target host for native parasites. Mar Ecol Prog Ser 636:91–106

Blakeslee AMH, Ruocchio M, Moore CS, Keogh CL (2020b) Altered susceptibility to trematode infection in native versus introduced populations of the European green crab. Aquat Invasions 15:177–195

Bojko J, Stebbing PD, Dunn AM, Bateman KS and others (2018) Green crab *Carcinus maenas* symbiont profiles along a North Atlantic invasion route. Dis Aquat Org 128:147–168

Brawley SH, Coyer JA, Blakeslee AM, Hoarau G and others (2009) Historical invasions of the intertidal zone of Atlantic North America associated with distinctive patterns of trade and emigration. Proc Natl Acad Sci USA 106:8239–8244

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Cai R, Kayal E, Alves-de-Souza C, Bigeard E and others (2020) Cryptic species in the parasitic *Amoebophrya* species complex revealed by a polyphasic approach. Sci Rep 10:2531

Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J Biogeogr 30:1809–1820

Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. Science 261:78–82

Cho SM (2012) Development of denaturing high-performance liquid chromatography (DHPLC) assay for parasite infection in grass shrimp, *Palaemonetes pugio*. Fish Aquat Sci 15:107–115

Dima JB, de Vido NA, Leal GA, Baron PJ (2009) Fluctuations in the biochemical composition of the Patagonian stone crab *Platyxanthus patagonicus* A. Milne Edwards, 1879 (Platyxanthidae: Brachyura) throughout its reproductive cycle. Sci Mar 73:423–430

Dumas JV, Witman JD (1993) Predation by herring gulls (*Larus argentatus* Coues) on two rocky intertidal crab species [*Carcinus maenas* (L.) & *Cancer irroratus* Say]. J Exp Mar Biol Ecol 169:89–101

Epifanio CE (2013) Invasion biology of the Asian shore crab *Hemigrapsus sanguineus*: a review. J Exp Mar Biol Ecol 441:33–49

France SC, Rosel PE, Ewann J (1996) DNA sequence variation of mitochondrial large-subunit rRNA. Mol Mar Biol Biotechnol 5:15–28

Freeman AS, Frischeisen A, Blakeslee AM (2016) Estuarine fouling communities are dominated by nonindigenous species in the presence of an invasive crab. Biol Invasions 18:1653–1665

Galaktionov KV, Blasco-Costa I, Olson PD (2012) Life cycles, molecular phylogeny and historical biogeography of the '*pygmaeus*' microphallids (Digenea: Microphallidae): widespread parasites of marine and coastal birds in the Holarctic. Parasitology 139:1346–1360

Giulietti L, Karlsbakk E, Cipriani P, Shayo SD, Storesund JE, Levsen A (2020) Molecular characterization of the myoliquefactive fish parasite *Kudoa mirabilis* (Cnidaria, Kudoidae) from SW Indian Ocean and its phylogenetic relationship with the *Kudoa thyrsites* species complex. Microorganisms 8:1352

Goedknegt MA, Feis ME, Wegner KM, Luttikhuizen PC and others (2016) Parasites and marine invasions: ecological and evolutionary perspectives. J Sea Res 113:11–27

Goedknegt MA, Havermans J, Waser AM, Luttikhuizen PC and others (2017) Cross-species comparison of parasite richness, prevalence, and intensity in a native compared to two invasive brachyuran crabs. Aquat Invasions 12: 201–212

Good TP (1998) Great black-backed gull (*Larus marinus*). In: Poole A, Gill F (eds) The birds of North America, No. 330. The Birds of North America, Philadelphia, PA, p 1–32

Griffen BD, Altman I, Hurley J, Mosblack H (2011) Reduced fecundity by one invader in the presence of another: a potential mechanism leading to species replacement. J Exp Mar Biol Ecol 406:6–13

Griffen BD, Altman I, Bess BM, Hurley J, Penfield A (2012) The role of foraging in the success of invasive Asian shore crabs in New England. Biol Invasions 14:2545–2558

Guégan JF, Kennedy CR (1993) Maximum local helminth parasite community richness in British freshwater fish: a test of the colonization time hypothesis. Parasitology 106: 91–100

Hamid A, Batu DTL, Riani E, Wardiatno Y (2016) Reproductive biology of blue swimming crab (*Portunus pelagicus* Linnaeus, 1758) in Lasongko Bay, Southeast Sulawesi-Indonesia. Aquacult Aquarium Conserv Legis 9:1053–1066

Heard RW, Overstreet RM (1983) Taxonomy and life histories of two North American species of "*Carneophallus*" (= *Microphallus*) (Digenea: Microphallidae). Proc Helminthol Soc Wash 50:170–174

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Hunter WS, Vernberg WB (1953) Early stages in the life cycle of the trematode, *Gynaecotyla adunca* (Linton, 1905). Trans Am Microsc Soc 72:163–170

Huspeni TC (2000) A molecular genetic analysis of host specificity, continental geography, and recruitment dy-

namics of a larval trematode in a salt marsh snail. PhD dissertation, University of California, Santa Barbara, CA

James BL (1968) The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. Field Stud 2:615–650

Jensen MP, Allen CD, Eguchi T, Bell IP and others (2018) Environmental warming and feminization of one of the largest sea turtle populations in the world. Curr Biol 28: 154–159

Johnson PTJ, Thieltges DW (2010) Diversity, decoys and the dilution effect: how ecological communities affect disease risk. J Exp Biol 213:961–970

Jones SJ, Lima FP, Wethey DS (2010) Rising environmental temperatures and biogeography: poleward range contraction of the blue mussel, *Mytilus edulis* L., in the western Atlantic. J Biogeogr 37:2243–2259

Jungblut S, McCarthy ML, Boos K, Saborowski R, Hagen W (2018) Seasonal lipid storage and dietary preferences of native European versus invasive Asian shore crabs. Mar Ecol Prog Ser 602:169–181

Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spillback: a neglected concept in invasion ecology? Ecology 90:2047–2056

Kennish R (1997) Seasonal patterns of food availability: influences on the reproductive output and body condition of the herbivorous crab *Grapsus albolineatus*. Oecologia 109:209–218

Keogh CL, Miura O, Nishimura T, Byers JE (2017) The double edge to parasite escape: invasive host is less infected but more infectable. Ecology 98:2241–2247

Kołodziej-Sobocińska M, Brzeziński M, Niemczynowicz A, Zalewski A (2018) High parasite infection level in non-native invasive species: it is just a matter of time. Ecography 41:1283–1294

Krakau M, Thieltges DW, Reise K (2006) Native parasites adopt introduced bivalves of the North Sea. Biol Invasions 8:919–925

Kroft KL, Blakeslee AMH (2016) Comparison of parasite diversity in native panopeid mud crabs and the invasive Asian shore crab in estuaries of northeast North America. Aquat Invasions 11:287–301

Kyomo J (1988) Analysis of the relationship between gonads and hepatopancreas in males and females of the crab *Sesarma intermedia*, with reference to resource use and reproduction. Mar Biol 97:87–93

Laverty C, Nentwig W, Dick JT, Lucy FR (2015) Alien aquatics in Europe: assessing the relative environmental and socioeconomic impacts of invasive aquatic macroinvertebrates and other taxa. Manag Biol Invasions 6:341–350

Lehnert SJ, DiBacco C, Jeffery NW, Blakeslee AM and others (2018) Temporal dynamics of genetic clines of invasive European green crab (*Carcinus maenas*) in eastern North America. Evol Appl 11:1656–1670

Littlewood DT, Olson PD (2001) Small subunit rDNA and the Platyhelminthes: signal, noise, conflict and compromise. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor & Francis, London, p 262–278

Lord JP, Williams LM (2017) Increase in density of genetically diverse invasive Asian shore crab (*Hemigrapsus sanguineus*) populations in the Gulf of Maine. Biol Invasions 19:1153–1168

Marcogliese DJ, Cone DK (1997) Food webs: a plea for parasites. Trends Ecol Evol 12:320–325

Matheson K, Gagnon P (2012) Temperature mediates non-competitive foraging in indigenous rock (*Cancer irroratus* Say) and recently introduced green (*Carcinus maenas* L.) crabs from Newfoundland and Labrador. J Exp Mar Biol Ecol 414:6–18

McDermott JJ (1998) The western Pacific brachyuran (*Hemigrapsus sanguineus*: Grapsidae), in its new habitat along the Atlantic coast of the United States: geographic distribution and ecology. ICES J Mar Sci 55:289–298

Minchin D, Gollasch S, Cohen AN, Hewitt CL, Olenin S (2009) Characterizing vectors of marine invasion. In: Rilov G, Crooks JA (eds) Biological invasions in marine ecosystems. Springer, Berlin, p 109–116

Miura O, Kuri AM, Torchin ME, Hechinger RF, Dunham EJ, Chiba S (2005) Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Crosse). Int J Parasitol 35:793–801

Miura O, Torchin ME, Kuris AM, Hechinger RF, Chiba S (2006) Introduced cryptic species of parasites exhibit different invasion pathways. Proc Natl Acad Sci USA 103: 19818–19823

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ 6:485–492

Poulin R (2011) Evolutionary ecology of parasites. Princeton University Press, Princeton, NJ

Pung OJ, Grinstead CB, Vives SP (2006) Variation in the geographic and temporal distribution of *Microphallus turgidus* (Trematoda: Microphallidae) in grass shrimp (*Palaemonetes* spp.) on tidal rivers in southeast Georgia, USA. Comp Parasitol 73:172–178

Pung OJ, Burger AR, Walker MF, Barfield WL, Lancaster MH, Jarrous CE (2009) *In vitro* cultivation of *Microphallus turgidus* (Trematoda: Microphallidae) from metacercaria to ovigerous adult with continuation of the life cycle in the laboratory. J Parasitol 95:913–919

Reisinger LS, Lodge DM (2016) Parasites alter freshwater communities in mesocosms by modifying invasive crayfish behavior. Ecology 97:1497–1506

Rohde K (ed) (2005) Marine parasitology. CSIRO Publishing, Wallingford

Roman J (2006) Diluting the founder effect: Cryptic invasions expand a marine invader's range. Proc R Soc B 273: 2453–2459

Ross JL, Ivanova ES, Severns PM, Wilson MJ (2010) The role of parasite release in invasion of the USA by European slugs. Biol Invasions 12:603–610

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. Am Zool 37:621–632

Seebens H, Schwartz N, Schupp PJ, Blasius B (2016) Predicting the spread of marine species introduced by global shipping. Proc Natl Acad Sci USA 113:5646–5651

Seebens H, Blackburn TM, Dyer EE, Genovesi P and others (2017) No saturation in the accumulation of alien species worldwide. Nat Commun 8:14435

Stunkard HW (1983) The marine cercariae of the Woods Hole, Massachusetts region, a review and a revision. Biol Bull (Woods Hole) 164:143–162

Thieltges DW, Reise K, Prinz K, Jensen KT (2009) Invaders interfere with native parasite–host interactions. Biol Invasions 11:1421–1429

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Front Ecol Environ 2: 183–190

Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol Invasions 3:333–345

Torchin ME, Lafferty KD, Kuris AM (2002) Parasites and marine invasions. Parasitology 124:137–151

Van Buskirk J, Mulvihill RS, Leberman RC (2009) Variable shifts in spring and autumn migration phenology in North American songbirds associated with climate change. Glob Change Biol 15:760–771

West J, Mitchell A, Pung OJ (2014) Optimization of conditions for *in vitro* culture of the microphallid digenean *Gynaecotyla adunca*. J Parasitol Res 2014:382153

Wolff WJ, Reise K (2002) Oyster imports as a vector for the introduction of alien species into northern and western European coastal waters. In: Leppäkoski E, Gollasch S, Olenin S (eds) Invasive aquatic species of Europe. Distribution, impacts and management. Springer, Dordrecht, p 193–205

Zetlmeisl C, Hermann J, Petney T, Glenner H, Griffiths C, Taraschewski H (2011) Parasites of the shore crab *Carcinus maenas* (L.): implications for reproductive potential and invasion success. Parasitology 138:394–401

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