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Research Article

Altered susceptibility to trematode infection in native versus introduced populations of the European green crab

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Abstract

Non-indigenous species (NIS) often experience a reprieve from infection by their co-evolved indigenous parasite load when they invade novel regions. Yet absolute escape from parasites may be precluded (notably with time) by introductions of parasites from the native range, by novel parasite acquisitions in the non-indigenous range, or both. As a result, NIS infection susceptibility may differ or change in indigenous versus novel regions due to divergent coevolutionary histories and parasite selective pressures – thereby influencing host resistance and/or compatibility. To investigate this question, we reciprocally exposed native (Europe) and non-native (eastern North America) individuals of the globally-invasive green crab, *Carcinus maenas*, to trematode parasites from both regions. We found that infection susceptibility differed by parasite origin: native European crabs exposed to native European parasites had lower infection prevalence and abundance, and the lowest proportion of irregular trematode metacercarial cysts or encapsulated metacercarial cysts (due to host immunity), compared with a treatment demonstrating possible prior coevolutionary history (~ 200 years ago) between the host and its parasites (invaded North American crabs exposed to native European parasites). Metacercarial cyst abundance was higher in the treatment with little to no coevolutionary history between host and parasite (native European crabs exposed to native North American trematodes) compared to a treatment with recent association in the last two centuries (invasive North American crabs exposed to native North American parasites). Our study provides further evidence that infection susceptibility can differ depending on coevolutionary history, which may be rapidly influenced by altered parasite selection pressures. It also provides greater understanding of the impact of human-mediated introductions on the coevolutionary dynamics of organisms worldwide.

Key words: *Carcinus maenas*, coevolutionary history, evolved defense, infection, non-indigenous species, parasite

Introduction

When species are introduced to new regions, they may leave behind co-evolved natural enemies, like parasites (Torchin and Lafferty 2009). Following invasions, hosts may enjoy a substantial reduction in parasite richness and diversity in non-native versus native ranges – a robust signature across systems even when accounting for the invader's specific

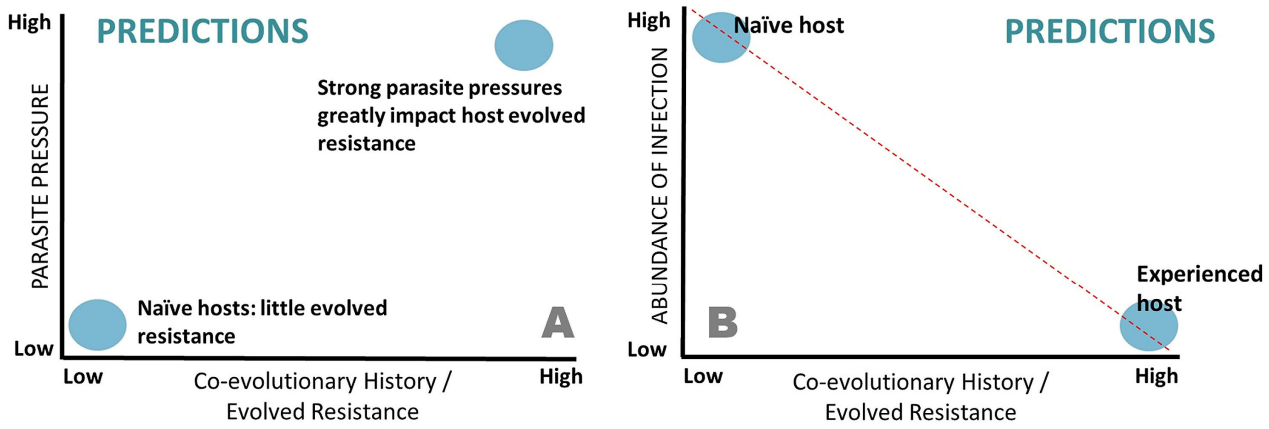

Figure 1. Theoretical predictions for coevolutionary history and the evolution of parasite resistance (low versus high) depending on: (A) parasite pressure (low versus high), whereby hosts under strong parasite pressures and long-term coevolutionary history are likely to evolve resistance to parasitism versus a naïve host; and (B) infection abundance, which is expected to show low infection under long-term coevolutionary history and high evolved resistance (i.e., an "experienced" host) versus a "naïve" host, which may have limited evolved resistance and therefore demonstrate higher parasite abundance.

source region (Torchin et al. 2003; Blakeslee 2016). On average, introduced hosts in marine systems possess about half their native parasite loads (Blakeslee et al. 2013). This "parasite escape" could be a contributing factor to an introduced host's success in novel locations where there is no prior coevolutionary history (Torchin and Mitchell 2004; Prenter et al. 2004; Torchin and Lafferty 2009; Goedknegt et al. 2017; Keogh et al. 2017). Due to strong influences on host ecological and evolutionary trajectories (Alvarez et al. 1995; Meiβner and Bick 1999; Mouritsen and Poulin 2002; Lafferty and Kuris 2009), a release from native parasites may greatly influence the demographic success of some introduced species, resulting in larger individual sizes and potential competitive advantages in non-native versus native ranges (Torchin et al. 2001; Wolfe 2002; Aliabadi and Juliano 2002; Parker et al. 2013). Moreover, because defense against parasitic infection can be physiologically and energetically costly (Sheldon and Verhulst 1996; Moret and Schmid-Hempel 2000; Sadd and Schmid-Hempel 2009; Ardia et al. 2012), hosts that escape coevolved parasites may benefit by re-allocating "defense" resources (plastically or across generations) to traits such as growth or reproduction (Blossey and Notzold 1995; Perkins et al. 2008; White and Perkins 2012). Yet this redirection of resources could incur a long-term cost to non-native host populations if reduced immune defense investment leaves a host more susceptible (i.e., naïve) to infection in future encounters with its coevolved parasites or with novel parasites (White and Perkins 2012). Thus, this relaxation of strong parasite pressures may lessen a host's resistance to infection with time, thereby enhancing its infection susceptibility in future encounters (Figure 1). For example, non-native populations of the Asian shore crab (*Hemigrapsus sanguineus*) in northeastern USA showed higher susceptibility to infection by native castrating rhizocephalan parasites from Japan than crabs sourced from the native range where parasite selection pressure was consistently high

(Keogh et al. 2017). Coevolutionary history is therefore predicted to be a strong driver of parasite prevalence and diversity patterns when viewed across multiple host populations of varying evolutionary histories.

Of the major metazoan parasite taxa that have been globally transported with anthropogenic vectors, digenean trematodes are one of the most commonly detected parasite groups in native and non-native populations (Blakeslee et al. 2013). This ubiquitous parasite group links diverse host assemblages through complex life cycles (Rohde 2005). Digenean trematodes typically infect 2–3 hosts to complete life cycles, most commonly using a gastropod as first-intermediate host. At this stage, trematodes go through asexual reproduction and intermittently release hundreds to thousands of larval cercariae into the water column which then seek (in many cases) a second-intermediate host, where they encyst as metacercariae. The life cycle is completed when the final host consumes the second-intermediate host via trophic transmission (Combes et al. 1994; Lafferty 1999). The spatial distribution of infective trematode larvae can be heterogeneous over a geographic area depending on the presence and abundance of intermediate and definitive hosts, but can also be temporally consistent, creating patches of high and low infection risk for both first and second intermediate hosts (Fredensborg et al. 2006; Byers et al. 2008, 2016).

Digenean trematodes can be strong selective forces for intermediate hosts, particularly in gastropods where infection often results in castration (e.g., Sousa 1983). For example, evidence of differential defense investment based on historical infection risk was found in two *Littorina* spp. snails with local reproduction, but not a third species with pelagic larval dispersal. In that study, snails from sites with historically high trematode infection risk showed significantly lower susceptibility to lab-induced infection than snails sourced from sites with historically low infection risk, suggesting local adaptation of host defense investment (Keogh et al. 2015). While fitness consequences to second-intermediate hosts are often less severe than in upstream gastropod hosts, evidence from multiple species suggests that local adaptation may still play a role in the evolution of host defense. For example, in the amphipod *Paracalliope novizealandiae*, hosts originating from habitats with consistently high trematode infection risk had both lower infection intensity and higher rates of encapsulation of the trematode *Maritrema novaezealandensis* than amphipods from a low infection risk site in experimental lab infections (Bryan-Walker et al. 2007). Similarly, sticklebacks (*Gasterosteus aculeatus*) from lake habitats with high abundances of the eye fluke *Diplostomum pseudospathaceum* had higher immune responsiveness and lower metacercarial infection intensity than sticklebacks from rivers where trematode infection risk was low (Kalbe and Kurtz 2006). Moreover, several studies have demonstrated that behavioral manipulation of second-intermediate hosts enhance trophic transmission to the final host, thereby completing the parasite life cycle

(Lafferty and Morris 1996; Lafferty 1999; Poulin 2010; Thomas et al. 2010). Because behavioral manipulations could ultimately enhance mortality in the second-intermediate host, this may exert pressure on intermediate hosts to adapt defenses, particularly if trematode prevalence and intensity are high and hosts become infected early in life.

Here, we investigated the influence of coevolutionary history and species introduction on prevalence and abundance of digenean trematode metacercariae in the globally-invasive European green crab (*Carcinus maenas*), focusing on its native European range and its oldest introduced range in eastern North America. The crab invaded this latter region in the early 1800s likely with solid ballast in ships coming from southwestern Europe to eastern North America (Carlton and Cohen 2003; Roman 2006). In both its native and non-native regions, the crab is infected by microphallid trematodes that utilize *Littorina* spp. snails as upstream hosts and shorebirds (typically seagulls) as final hosts (Blakeslee et al. 2015). One microphallid trematode frequently identified in *C. maenas* in both its native and non-native regions is *Microphallus similis* (James 1968; Pohley 1976; Blakeslee et al. 2009, 2015). This trematode can be morphologically identified to species level in upstream snail hosts utilizing published keys and descriptions (James 1968). In prior work, *M. similis* has been found to influence *C. maenas* righting and foraging behaviors, as well as its immune response, particularly shortly after infection (Blakeslee et al. 2015). To date, it is still unclear how the invasion of *C. maenas* may have influenced evolved resistance to trematode parasitism in its native versus non-native region. Our study investigated this question utilizing a common garden experiment that exposed crab hosts from native (Europe) and non-native (North America) regions to the trematode *M. similis*, also sourced from these two regions. We then measured infection prevalence and metacercarial cyst abundance in crabs post-exposure.

Materials and methods

Crab and Snail Sampling

In summer 2016, we sampled *Carcinus maenas* crabs and *Littorina* spp. periwinkle snails (primarily *L. saxatilis* and *L. obtusata*, and some *L. littorea*) for the exposure experiment from the native European range and the non-native northeastern North American range. In the native range, crabs were collected from a site in the Netherlands (Royal Netherlands Institute for Sea Research (NIOZ) in Texel) where prior research found relatively low infection prevalence of microphallid trematodes in *C. maenas* crabs (Goedknegt et al. 2017), including a recent 2018 screening by members of our group (and additionally, D. Thieltges (NIOZ) and P. Luttikhuizen (NIOZ)) that found relatively low metacercarial abundance (average of 28 cysts per crab) and an infection prevalence of 50% (n = 10 crabs) (Blakeslee

unpublished). In the non-native northeastern North America range, crabs were collected from southern New England and mid-Atlantic USA sites (Durham, New Hampshire and Goldsmith, Long Island, New York) representing the historical location of the crab's introduction (Roman 2006; Darling et al. 2014); to date, these sites remain genetically similar to the original founding population (Lehnert et al. 2018). In addition, prior research (Blakeslee et al. 2009, 2015; Blakeslee *unpublished*) revealed low microphallid infection prevalence and abundance in *C. maenas* from these sites. For example, during a 2016 survey from the Goldsmith site, trematode prevalence in *C. maenas* was 26%, with an average abundance of one metacercarial cyst per crab (n = 15 crabs) (Blakeslee, *unpublished*). Moreover, prior sampling in 2012 from the Durham, NH site (Blakeslee et al. 2015) had similar results with 32% prevalence of metacercarial cysts and an average abundance of 0.5 metacercarial cysts per crab (n = 35 crabs). Thus, our native and non-native sites were intentionally chosen to select sites with low *Microphallus similis* infection prevalence and abundance, thereby limiting the abundance of pre-existing cysts in our treatment groups (*a priori* knowledge of infection status for crabs used in the experiment is not possible because it requires destructive sampling).

In contrast, we collected upstream snail hosts (*Littorina* spp.) from native and non-native sites where microphallid trematode prevalence was at moderate to higher levels. In Europe, snails were collected from a location (Esbjerg) in southern Denmark where prior research (Blakeslee and Byers 2008) found 20% trematode infection prevalence. This site was also chosen because it is part of the Wadden Sea that links the Netherlands, Germany, and southern Denmark within the North Sea, such that our sampled crabs and snails were part of the same regional system within the native range. Our upstream *Littorina* spp. snail hosts came from a New England site (York, southern Maine) where trematode infection prevalence was ~ 13% (Blakeslee and Byers 2008; Byers et al. 2016; Blakeslee *unpublished*).

One limitation in our sampling of crabs and snails from the Netherlands and Denmark, respectively, is that there are genetic differences between southwestern and northern populations of *C. maenas*, with a genetic break between the Netherlands and Germany (Roman and Palumbi 2004). Based on genetic data, southwestern populations are the likely source for the original 1800s US introduction of *C. maenas* (Roman 2006). As a result, our European collection sites may be just outside of the original source region of crabs to the USA, thus possibly making the North American invaded crabs relatively naïve to trematodes sourced from the Wadden Sea. Yet, given that microphallid trematodes use dispersive, mobile shorebirds as common definitive hosts, gene flow could be more well-mixed in these trematode populations than their crab hosts. With our current genetic data, however, we cannot discern whether similar genetic differences exist in northern versus southwestern trematodes compared to their hosts. As

such, we were cautious in our characterization of the treatment where North American invasive crabs were exposed to native European trematodes.

In total, 15–20 crabs were collected per site using a combination of hand collecting and trapping, and 500 snails (200 *L. obtusata*, 200 *L. saxatilis*, 100 *L. littorea*) were collected per site by hand in the low intertidal zone. We focused collections on *L. obtusata* and *L. saxatilis* as these snails are more likely to host *M. similis* (James 1968; Blakeslee and Byers 2008), and thus would ensure that we had a sufficient abundance of microphallid trematodes to induce infection in our downstream crab hosts (based on methodologies in Blakeslee et al. 2015). From Europe, crabs and snails were collected by CLK and shipped two-day mail to our common garden facility at East Carolina University in inland eastern North Carolina. From North America, crabs were collected by AMHB and driven by car to our NC facility (drive time was also approximately two days, and crabs were housed in aerated seawater during that time). After the experiment, all crabs and snails were euthanized and properly disposed of in waste receptacles at East Carolina University. This facility is located far inland of locations suitable for crab or snail survival (it is also further south than any reported established populations of either the crab or the snails).

Exposure Treatments

Per above, the experimental crabs came from two different sources: native Europe and non-native northeastern North America. In a common garden experiment, crabs from each source region were randomly assigned to treatments and exposed to snails from the two parasite sources. The four treatment groups were as follows: (I) EU-EU, native European trematodes and native European crabs representing long-term coevolutionary history between the parasite and its host; (II) EU-NA, native European trematodes and invaded North American crabs, representing a host that may have shared prior coevolutionary history with the parasites but has not experienced them for ~ 200 years; (III) NA-NA, native North American trematodes and non-native North American crabs, representing native North American trematodes and non-native crabs that have short-term history with the crab of < 200 years; and (IV) NA-EU, native North American trematodes and native European crabs, representing no coevolutionary history between trematode and host within the recent evolutionary past.

Exposure Experiment

Prior to the start of the study, crabs were acclimatized to laboratory conditions for 48 hours. Crabs sourced from Europe were then assigned to either the European trematode exposure treatment group or the North American trematode exposure group; likewise, crabs sourced from North America were assigned to either the European or North American

exposure groups. For the European trematode exposure group, 8 individuals were assigned to the EU-EU treatment and 9 individuals assigned to the NA-EU treatment. During the 4 week post-exposure period, one crab died in the EU-EU group, resulting in 7 EU-EU crabs and 9 NA-EU crabs. For the North American trematode exposure group, 17 individuals were assigned to the NA-NA treatment and 15 to the NA-EU treatment. One of these latter crabs died during the 4 week post-exposure period, resulting in 14 total crabs in that treatment. Across all treatments, crab size ranged from 34.8 mm to 77.6 mm carapace width (CW) (average = 56.0 mm). Twenty-five crabs were males (57.9 mm ± 9.8 CW) and 22 were females (53.5 mm ± 9.9 CW). Crab size and sex were kept as consistent as possible during assignments for the treatments.

In order to keep track of the source region for each crab and to prevent cannibalism, crabs were placed into separate, labeled housing units. These small plastic cages allowed for water flow and did not impede trematode cercariae from swimming through. These individual cages were then submerged and allowed to float (i.e., they were not fixed) in two large (45 L) plastic bins serving as the common garden arenas for each trematode exposure source (Europe or North America). These bins were filled ¾ of the way with artificial seawater, made from deionized water and Instant Ocean, at 30 PSU (see Supplementary material Figure S1 for schematic of experimental set-up).

Cercarial emergence from snail hosts occurs more frequently following a desiccation period, similar to what would occur naturally during tides (Combes et al. 1994; Blakeslee et al. 2015). Therefore, snails from each exposure source (Europe or North America) were divided into two batches (n = 125 each, with the number of snails among the three *Littorina* species evenly divided per batch) that were exchanged every 12 hours. At any given time, one batch would be submerged in the experimental arena to release cercariae, while the other was set aside to desiccate in a cool location. Snails in each submerged batch were further divided in half and placed into two metal strainers covered by a fine mesh that allowed cercariae to emerge into the water. Because shed *M. similis* swim downwards (e.g., McCarthy et al. 2002), the strainers were spaced evenly apart on the left and right sides of the bin, attached to its top, such that each strainer was submerged just below the water line (see Figure S1). We chose this method of induced infection (i.e., natural emergence from upstream snail hosts in a common garden setting) in order to expose sentinel European and North American crabs to similar levels and genotypes of infective cercariae within each experimental arena. Moreover, crabs were randomly distributed in the exposure bins and were mixed around, such that crabs would have relatively similar encounter likelihoods by emerging cercariae. Thus all crabs within the same bin were exposed to the same pool of potentially infective cercariae during the exposure period.

Every 12 hours, snail batches were swapped (submerged or desiccated) to enhance cercarial emergence and infection likelihood in crab hosts. Cercarial emergence was enumerated once daily (0900h) by pooling five 1-mL water samples from the four corners and center of the bin and systematically scanning for all cercariae (but particularly *M. similis*) under a stereomicroscope at 4x power (10x oculars). Crabs were exposed to snails in the two treatment bins for 72 hours. Previously, this length of time has been sufficient to experimentally infect crabs and obtain a moderate infection intensity (100s of metacercarial cysts per individual crab) (Blakeslee et al. 2015). Following the exposure period, each crab was moved to an individual, labeled plastic aquarium (Lees Kritter Keeper – Small, 9.1"L × 6" W × 6.6"H) with aerated artificial seawater (30 PSU) and placed into an incubator at 20 °C (12:12 light:dark cycle) for four weeks. During that time, crabs were fed a mixed diet of *Ulva lactuca* and TetraFin goldfish flakes *ad libidum*, and water was changed weekly. This four week post-exposure "incubation" period was to ensure sufficient time for trematode metacercarial cysts to demonstrate characteristic morphology for identification (Stunkard 1957; Blakeslee et al. 2015). At the end of the experiment, we dissected a subset (n = 100) of the snails used in the exposure experiment and found a 3% microphallid trematode infection prevalence in both the European snails and the snails from northeastern North America.

Dissections, Prevalence, and Abundance Quantification

Following the 4-week post-exposure period, all crabs were simultaneously euthanized by freezing in a 0 °C chest freezer. This ensured that newly formed metacercarial cysts would be preserved for cyst counts and abundance data. Crabs were measured and sexed and then dissected by separating the upper from the lower carapace (see methods in Blakeslee et al. 2009). To obtain metacercarial cyst counts, tissues from the following regions of the body were extracted via a series of standardized tissue samples ("snips"): the hepatopancreas (energy storage tissue) (6 snips), thoracic ganglion (nerve center) (1 snip), and gonad (1 snip). Tissue snips focused on the hepatopancreas because prior work found that metacercarial cysts concentrated in this region (Torchin et al. 2001; Blakeslee et al. 2009, 2015). The amount of tissue per snip was 22 × 22 mm (i.e., the size of a standard glass cover slip). Extracted tissue was squashed between the slide and cover slip to make metacercarial cysts easily visible under microscope illumination. Each slide was viewed under a compound microscope at 4x (with 10x oculars), and microphallid trematode metacercarial cysts were enumerated using a handheld tally counter. During these counts, we noted that while many metacercarial cysts were intact and spherical, some metacercarial cysts were irregularly shaped or demonstrated encapsulation and melanization (i.e., "brown bodies" representing host immune response; the metacercarial cyst was still detectable by shape and size after the host

melanization) (Figure S2). We therefore recorded these categories of metacercarial cysts (intact, irregular, or brown body) during counts. Moreover, pre-existing metacercarial cysts (intensities all < 10 individuals in a single crab) were detected in a subset of crabs from Europe and North America (~ 11%); these cysts were distinguished from newly-formed, experimentally-induced metacercarial cysts by the presence of a thicker and distinct cyst wall (Figure S2). We have also observed that recently induced experimental infections are identifiable by the strong spatial aggregation of cysts with a very thin cyst wall (Blakeslee et al. 2015). Thus, though a proportion of experimental crabs had pre-existing cysts, the abundance was low and unlikely to significantly impact the results.

Across all crabs, the majority of metacercarial cysts were found in the hepatopancreas with very few occurrences in the other two tissues; therefore, analyses focused on the hepatopancreas only. While individual counts of cysts per crab provided a measure of raw abundance based on the six hepatopancreas tissue snips, cysts per gram of hepatopancreas was used as a standardized response variable. Metacercarial cyst abundance was defined as the number of metacercarial cysts per individual for all sampled individuals, including those crabs where no parasites were detected (i.e., the abundance measure also includes zero values) (Lafferty et al. 1997). Per crab, the number of cysts per gram of hepatopancreas was estimated by multiplying the average number of cysts per snip (averaged across the six hepatopancreas snips) by the differential for a gram of hepatopancreas tissue using a standardized tissue weight per snip (0.116 grams) based on prior data (n = 55) (Blakeslee et al. 2015).

Statistical Analyses

Because we could not control for differences in cercarial emergence (and therefore intensity of experimentally-induced infections) between the two exposure groups, we only made statistical comparisons within each exposure group (i.e., the European trematode exposure source treatments of EU-EU and EU-NA and the North American trematode exposure source treatments of NA-NA and NA-EU). We analyzed prevalence data using a generalized linear model with a binary distribution and logit link function, with infection status (infected = 1, uninfected = 0) as the response variable, and crab size (carapace width, mm), sex, and treatment as fixed effects. We analyzed abundance data using a generalized linear model with a poisson distribution and log link function, with cysts per gram hepatopancreas as the response variable, and treatment, size and sex as fixed effects. We conducted the following four analyses of metacercarial cyst abundance with this approach: (1) all metacercarial cysts; (2) intact metacercarial cysts only; (3) irregular metacercarial cysts only; (4) "brown bodies" only. To simplify presentation, the results of both common garden exposure groups were plotted on the same graphs. All statistical analyses were performed using JMP Pro 14.0 (SAS Institute, Inc.).

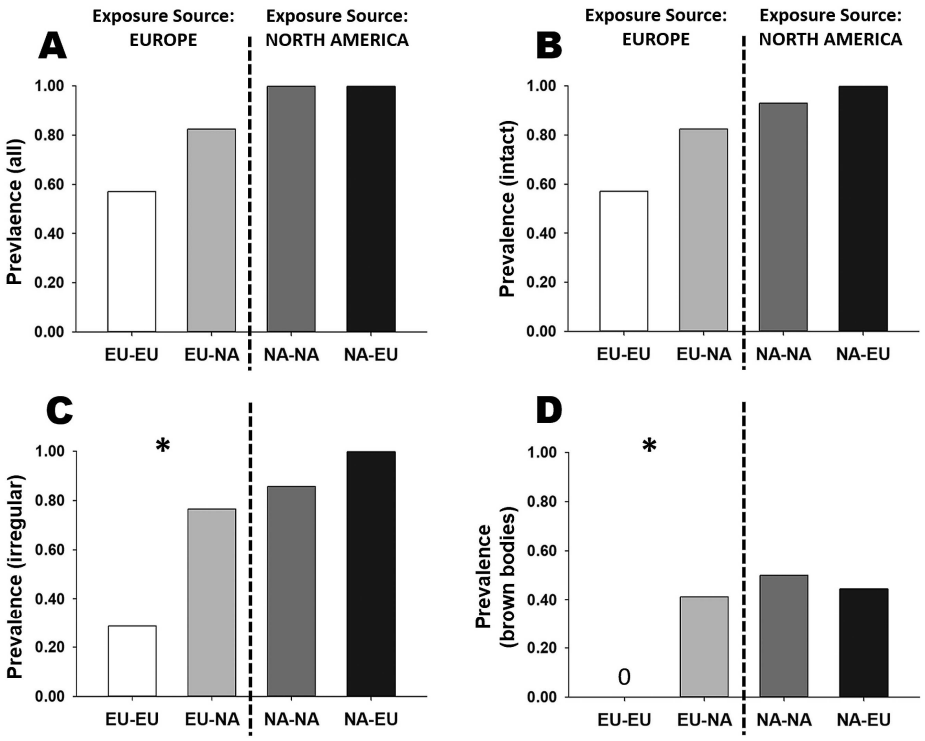

Figure 2. Prevalence of infection in *Carcinus maenas* for the four treatment groups, divided (dotted line) into crabs exposed to European (EU) trematodes and North American (NA) trematodes from upstream *Littorina* spp. hosts for all metacercarial cysts (A), intact metacercarial cysts (B), irregular metacercarial cysts (C), and brown bodies (D). * = marginal significance $p < 0.10$

Results

Infection Prevalence

European Trematode Source (EU-EU versus EU-NA)

For analyses of both all metacercarial cysts and intact metacercarial cysts (Figure 2A, B), there was no significant effect of treatment ($F = 2.58$, $p = 0.11$), sex ($F = 0.25$, $p = 0.62$), nor size ($F = 1.78$, $p = 0.18$) on infection prevalence. For the irregular metacercarial cysts, there was no significant effect of sex ($F = 0.56$, $p = 0.45$) nor size ($F = 0.04$, $p = 0.85$) on infection prevalence, but there was a marginally significant effect of treatment ($F = 3.13$, $p = 0.08$), with EU-NA having higher prevalence of irregular metacercarial cysts than EU-EU (Figure 2C). For brown body (melanized) cysts, there was no effect of sex ($F = 0.24$, $p = 0.62$) nor size ($F = 1.00$, $p = 0.32$) on infection prevalence, but there was a marginally significant effect of treatment ($F = 3.64$, $p = 0.06$), with EU-NA having higher prevalence of brown bodies than EU-EU (Figure 2D).

North American Trematode Source (NA-EU versus NA-NA)

For all metacercarial cysts, prevalence was 100% for both treatments, so there were no differences to analyze between them (Figure 2A). For intact, irregular and brown body metacercarial cysts, respectively, there was no

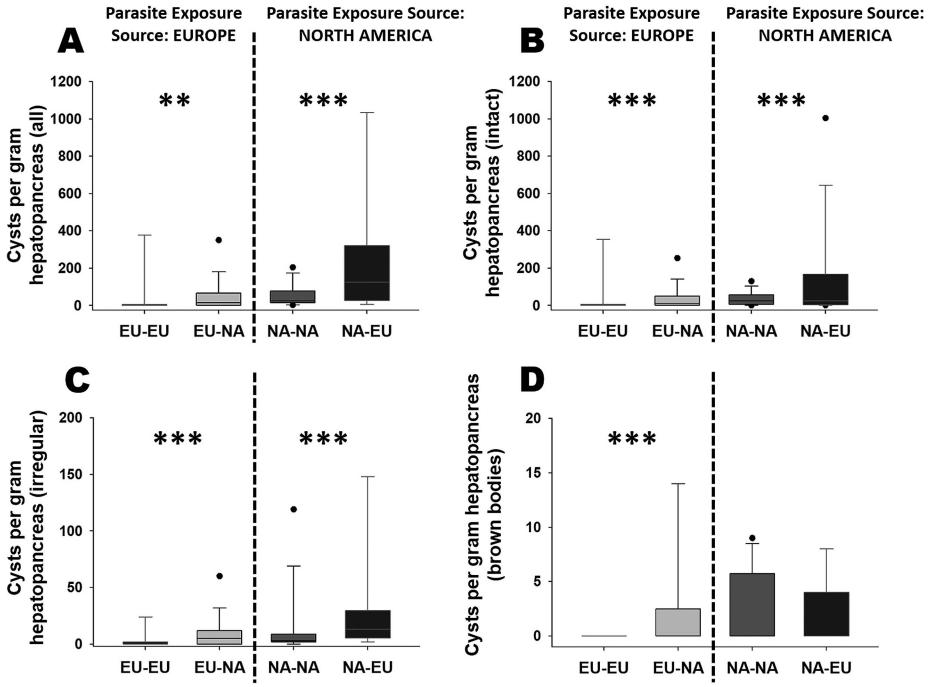

Figure 3. Abundance (cysts per gram hepatopancreas) of metacercarial cyst infection in the four treatments, divided (dotted line) into crabs exposed to European (EU) trematodes and North American (NA) trematodes from upstream *Littorina* spp. Hosts for (A) all metacercarial cysts, (B) intact metacercarial cysts only, (C) irregular metacercarial cysts only, and (D) brown bodies only. ** = $p < 0.01$, *** = $p < 0.001$.

effect of treatment [(intact) $F = 0.51$, $p = 0.47$; (irregular) $F = 2.19$, $p = 0.14$; (brown body) $F = 0.07$, $p = 0.80$)], size [(intact) $F = 1.53$, $p = 0.22$; (irregular) $F = 0.36$, $p = 0.55$; (brown body) $F = 2.35$, $p = 0.13$], nor sex [(intact) $F = 1.04$, $p = 0.31$; (irregular) $F = 0.24$, $p = 0.62$; (brown body) $F = 2.0$, $p = 0.16$] on infection prevalence (Figure 2B–D).

Abundance of Metacercarial Cysts

European Trematode Source (EU-EU versus EU-NA)

For all metacercarial cysts, there was a significant effect of both treatment ($F = 3.964$, $p = 0.047$) and sex ($F = 7.152$, $p = 0.008$) on metacercarial cyst abundance, with EU-NA having a higher abundance of cysts than EU-EU (Figure 3A) and females having higher abundances than males, but there was no effect of size ($F = 0.026$, $p = 0.850$). For intact metacercarial cysts, there was a significant effect of treatment ($F = 33.243$, $p < 0.001$) and sex ($F = 27.334$, $p < 0.001$) on metacercarial cyst abundance, with higher cyst abundance in EU-NA (Figure 3B) and females, but no effect of size ($F = 0.298$, $p = 0.585$). For the irregular metacercarial cyst analysis, there was a significant effect of treatment ($F = 30.653$, $p \leq 0.001$) and sex ($F = 6.531$, $p = 0.010$) on irregular cyst abundance, with EU-NA (Figure 3C) and males having higher irregular cyst abundance, but no effect of size ($F = 0.463$, $p = 0.496$). Finally, there was a significant effect of treatment ($F = 38.078$, $p < 0.001$), size ($F = 5.549$, $p = 0.019$), and sex ($F = 21.363$, $p < 0.001$) on the

abundance of brown body cysts, with EU-NA (Figure 3D), larger crabs, and males having greater abundances.

North American Trematode Source (NA-EU versus NA-NA)

For all metacercarial cysts, there was a significant effect of treatment ($F = 1090$, $p < 0.001$) and sex ($F = 95.942$, $p < 0.001$) on cyst abundance, with higher abundance in NA-EU (Figure 3A) and males, but no effect of size ($F = 2.088$, $p = 0.149$). For intact cysts, there was a significant effect of treatment ($F = 1171.594$, $p < 0.001$) and sex ($F = 71.635$, $p < 0.001$) but not size ($F = 1.897$, $p = 0.1684$) on intact metacercarial cyst abundance, with NA-EU (Figure 3B) and males having greater abundance. For irregular cysts, there was a significant effect of treatment ($F = 52.100$, $p < 0.001$), size ($F = 9.197$, $p = 0.002$) and sex ($F = 12.351$, $p < 0.001$) on cyst abundance, with NA-EU (Figure 3C), larger crabs, and males having higher irregular metacercarial cyst abundance. Finally, for brown body cysts, there was no significant effect of treatment ($F = 0.739$, $p = 0.390$) nor sex ($F = 0.560$, $p = 0.454$) on brown body abundance, but there was an effect of size ($F = 6.198$, $p = 0.013$), with larger crabs having greater abundance of brown body cysts.

Discussion

Host-parasite coevolutionary history is predicted to strongly drive parasite prevalence and diversity in hosts, particularly for impactful parasites that may significantly reduce a host's survival and reproduction. Hosts under long-term coevolutionary history and strong parasite selective pressures are more "experienced" and thus more likely to evolve resistance to parasitism when compared to hosts under low parasite pressure (i.e., "naïve" hosts), where energy allocation for defense is less useful (Figure 1). Generally, energy that is put towards defense can be costly and can also be diverted from important physiological functions and processes that would better enhance a host's survival and reproduction. Thus, in the absence or loss of such strong parasite pressures (e.g., when a non-indigenous species escapes native parasites), hosts may relax these defenses. Yet if put back in contact with the same or similar parasites, these relaxed hosts may be more prone to contracting infection compared to conspecifics in their coevolved range. Further, hosts that do not share recent coevolutionary history with generalist parasites might be more susceptible to infection than hosts that have evolved in lock-step with a locally occurring suite of parasites.

Our study examining reciprocal infection of trematode parasites in the native and non-native regions of the crab *Carcinus maenas* found patterns consistent with these hypotheses (Figure 4). When the trematode exposure source was North America, the "naïve" European crabs (NA-EU; no coevolutionary history) demonstrated a significantly higher abundance of metacercarial cysts when compared to non-native North American crabs

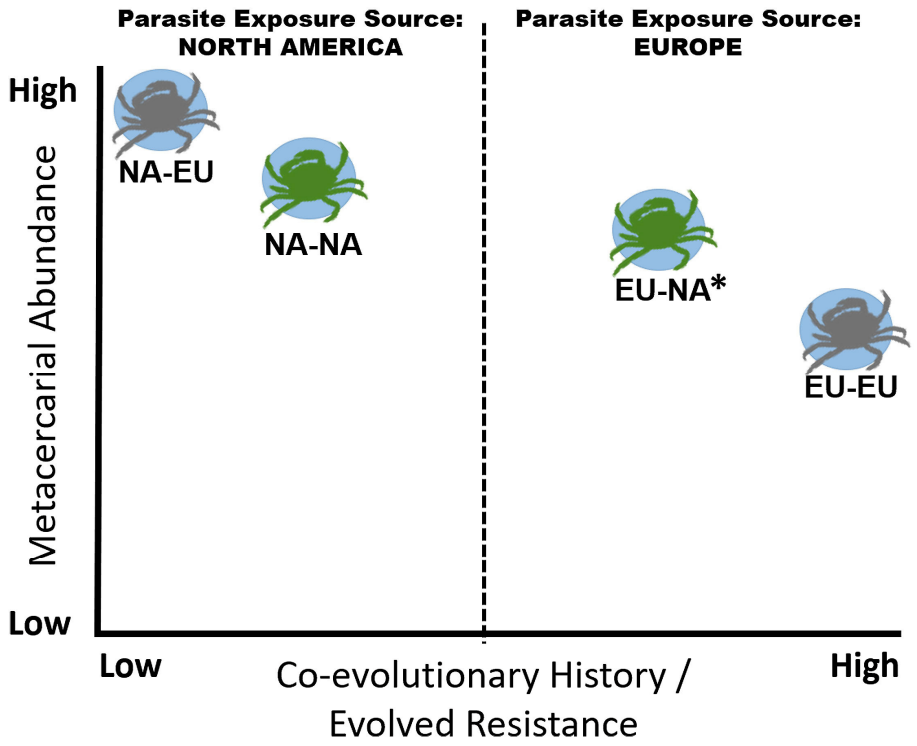

Figure 4. Outcomes of all four treatments in the common garden experiment based on coevolutionary history of the trematode parasite, *Microphallus similis*, and the crab host, *Carcinus maenas*. Crabs collected from the native European range are represented in grey, and crabs collected from the non-native North American range are represented in green. NA-EU = native European crabs exposed to North American trematodes, representing a group with no prior coevolutionary history; NA-NA = non-native North American crabs exposed to North American trematodes, representing a group with short-term coevolutionary history of < 200 years. EU-NA = non-native North American crabs exposed to European trematodes, representing a group with possible prior coevolutionary history. EU-EU = native European crabs exposed to European trematodes, representing coevolved host and parasite with long-term coevolutionary history. The dotted line demonstrates the statistical comparisons made within the trematode exposure groups of Europe and North America. * The asterisk represents uncertainty in EU-NA's placement along the coevolutionary history gradient (see Discussion).

(NA-NA), where association between host and parasite is < 200 years (Figure 3). When the trematode exposure source was Europe, the "experienced" European crabs (EU-EU; coevolved treatment) had lower prevalence and cyst abundance compared to non-native North American crabs (EU-NA) that may share prior coevolutionary history with European trematodes (Figures 2–3). However, as described in the methods, the invaded North American crabs represent a founding population that genetically differs from conspecifics in more northern populations (Roman and Palumbi 2004), and this is where we sourced our European trematodes. Thus if host and parasite patterns are congruent, the North American crabs may be relatively naïve to the European trematodes we exposed them to, thus affecting their status as having a prior shared coevolutionary history. A comprehensive genetic study of *Microphallus similis* in Europe is needed to determine the extent of trematode gene flow across European *C. maenas* populations. Regardless, the coevolved EU-EU treatment crabs demonstrated lower prevalence and abundance than the EU-NA treatment crabs, the latter having less experience with the trematode parasite.

Such differential prevalence and abundance in invaded versus native hosts can occur quite rapidly, as observed in another NIS shorecrab, *Hemigrapsus sanguineus*. This crab has been established in northeast North America for < 30 years; yet laboratory experiments have shown that invasive populations are more heavily parasitized by a native castrating rhizocephalan barnacle than native crab populations (Keogh et al. 2017). While similar, our study differs from this example because *C. maenas* is infected by microphallid trematodes in both native and non-native regions due to the presence of the same upstream *Littorina* spp. hosts in both locations. Microphallid trematodes infecting *C. maenas* in North America and Europe represent four genetically divergent lineages, and, based on genetic analyses, these appear to be different microphallid trematode species (Blakeslee et al. *in review*). Only one lineage infects *C. maenas* in Europe and matches genetically with the trematode species *Microphallus primas*, which uses European hydobiid snails as upstream hosts (James 1968). The other three lineages detected in North American *C. maenas* also infect a native North American crab (*Cancer irroratus*), and two of these lineages appear to be native North American trematode species that have host-switched to infect *C. maenas* in its introduced region. One lineage matched genetically with the trematode species *Gynaecotyla adunca*, which uses the mud snail *Tritia obsoleta* as upstream host (Hunter and Chait 1952), and the other lineage was closely related to the trematode species *M. turgidus*, which uses North American hydrobiid snails as upstream hosts (Blakeslee et al. *in review*). The fourth trematode species was identified as *M. similis*, which is cosmopolitan in Europe and North America in *C. maenas* and upstream *Littorina* spp. snails. To our knowledge, this is the only microphallid trematode found in both Europe and North America that uses *Littorina* spp. snails as upstream hosts and *C. maenas* as a downstream host. *Littorina* spp. snails (particularly *L. obtusata* and *L. saxatilis*) were the upstream hosts used in our investigation here. However, the presence of other microphallid trematodes in North America, along with the presence of *M. similis* in the crab's native and non-native ranges, may have influenced its infection susceptibility in North America because the crab was introduced to a region where microphallids were already present. For example, both North American trematode source treatments (NA-EU and NA-NA) had very high (100%) infection prevalence and abundance of metacercarial cysts following experimental exposure. Further, the NA-EU treatment, which represents no recent coevolutionary history between host and parasite (European crabs exposed to North American trematodes) had the highest abundance of metacercarial cysts. This may suggest that naïve crabs were less able to defend themselves against a parasite species that was already primed to infect them. The other North American exposure treatment (NA-NA), representing short-term association of host and parasite, demonstrated high infection prevalence but lower metacercarial

abundance than NA-EU, suggesting some level of evolved response to native North American trematodes over the past ~ 200 years.

In general, we found relatively high prevalence and abundance of metacercarial cysts across all treatments (with the exception of EU-EU). Past work has found little effect of trematode metacercarial cysts on *C. maenas* reproductive investment when compared to uninfected crabs (Blakeslee et al. 2015); thus fitness costs may be mild, lessening the selection for defense. Instead, selection may favor tolerance rather than resistance, as a result of conflicting selection pressures from a broader diversity of native-range parasites (Jokela et al. 2000; Baucom and de Roode 2011). However, in our study, we also revealed an effect of sex in some analyses, but the results were inconsistent with females having significantly higher metacercarial abundance in some analyses but males in others. These results could hint at some tradeoff that may exist between investment in reproduction and defense (e.g., French et al. 2007), but further investigation is warranted, particularly with higher sample sizes for each sex.

Interestingly, we discovered both irregular and melanized metacercarial cysts (i.e., "brown bodies") during our study (Figure S2). While the latter demonstrated little pattern by treatment, the irregular metacercarial cysts were most abundant in the NA-EU treatment where host and parasite had no coevolutionary history. In this treatment, irregular metacercarial cysts represented 12% of all the cysts in the infected crabs. This may suggest that though naïve hosts were more susceptible to infection than crabs with a more recent association (NA-NA), it may be more costly for the trematode to infect a less familiar host. At this point, it remains unclear whether these metacercarial cysts could still be viable upon reaching the final host. Yet their visibly distorted nature may suggest some level of impairment. Future studies could explore differences in excystment success and adult morphology in irregular versus intact metacercarial cysts (Hunter and Chait 1952; Irwin et al. 1984), as well as transmission success to final vertebrate hosts.

Conclusions

Our results are promising in detecting differences in parasite infection by coevolutionary history and inherent parasite pressures based on changing landscapes of hosts and parasites, which could be extended to multiple systems across terrestrial and aquatic environments. During this current period of anthropogenic global change, in which species are being moved around the globe at unprecedented rates (Ruiz et al. 1997; Seebens et al. 2013), the likelihood for hosts to escape their native parasite loads, along with new encounters of parasite lineages/species in novel environments, will continue to increase. Considering the highly influential role that many parasites play in these systems, these changes could have profound effects on the ecological and evolutionary trajectories of parasite and free-living

species around the world, as well as their interacting communities and ecosystems (Mouritsen and Poulin 2002; Lafferty and Kuris 2009; Viard et al. 2016). Our work suggests that we may also detect differential susceptibility based on invasion history in multiple introduced populations of *C. maenas* and perhaps other species across the globe. Here, we examined the green crab's oldest introduced location in northeastern North America (~ 200 years). Because *C. maenas'* patterns of parasite escape are influenced by time since introduction (Torchin and Lafferty 2009), expanding the current study would provide a more comprehensive comparison of the crab's native region to introduced populations from other areas around the world, which range in invasion age from decades (eastern and western North America, Japan, South Africa, and Argentina) to a couple centuries ago in eastern North America and Australia (Fofonoff et al. 2019). In fact, a large-scale follow-up investigation of multiple introduced regions of the crab, including global populations, will help further aid in our understanding of the effects of host-parasite coevolutionary history on parasite susceptibility, including recent coevolutionary history as a result of species invasion. Moreover, exploring these same questions in multiple global populations would further inform us of the impact of human-mediated introductions on the coevolutionary dynamics of organisms worldwide.

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Conflicts of interest

Dr. Blakeslee is part of the editorial team for the ICMB-X special issue. Dr. Blakeslee is also an Associate Editor for *Aquatic Invasions*.

Ethics

All animals were handled in accordance with University policy. As stated in the manuscript, all animals were properly euthanized following the experiment. Dr. Blakeslee is covered by a Scientific Collection Permit issued to East Carolina University (#706671).

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Schematic demonstration of experimental set-up.
Figure S2. Photographic representation of the cyst categories for the experiment.

This material is available as part of online article from:
http://www.reabic.net/aquaticinvasions/2020/Supplements/AI_2020_Blakeslee_etal_SupplementaryFigures.pdf