Research Article

Comparison of parasite diversity in native panopeid mud crabs and the invasive Asian shore crab in estuaries of northeast North America

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Abstract

Numerous non-indigenous species (NIS) have successfully established in new locales, where they can have large impacts on community and ecosystem structure. A loss of natural enemies, such as parasites, is one mechanism proposed to contribute to that success. While several studies have shown NIS are initially less parasitized than native conspecifics, fewer studies have investigated whether parasite richness changes over time. Moreover, evaluating the role that parasites have in invaded communities requires not only an understanding of the parasite diversity of NIS but also the species with which they interact; yet parasite diversity in native species may be inadequately quantified. In our study, we examined parasite taxonomic richness, infection prevalence, and infection intensity in the invasive Asian shore crab *Hemigrapsus sanguineus* De Haan, 1835 and two native mud crabs (*Panopeus herbstii* Milne-Edwards, 1834 and *Eurypanopeus depressus* Smith, 1869) in estuarine and coastal communities along the east coast of the USA. We also examined reproductive tissue allocation (i.e., the proportion of gonad weight to total body weight) in all three crabs to explore possible differences in infected versus uninfected crabs. We found three parasite taxa infecting *H. sanguineus* and four taxa infecting mud crabs, including a rhizocephalan castrator (*Loxothylacus panopaei*) parasitizing *E. depressus*. Moreover, we documented a significant negative relationship between parasite escape and time for *H. sanguineus*, including a new 2015 record of a native microphallid trematode. Altogether, there was no significant difference in taxonomic richness among the crab species. Across parasite taxa, *H. sanguineus* demonstrated significantly lower infection prevalence compared to *P. herbstii*; yet a multivariate analysis of taxa-specific prevalence demonstrated no significant differences among crabs. Finally, infected *P. herbstii* had the highest proportion of gonad weight to total body weight. Our study finds some evidence for lower infection prevalence in the non-native versus the native hosts. However, we also demonstrate that parasite escape can lessen with time. Our work has implications for the understanding of the potential influence parasites may have on the future success of NIS in introduced regions.

Key words: *Eurypanopeus depressus*, *Hemigrapsus sanguineus*, *Loxothylacus panopaei*, *Panopeus herbstii*, parasite escape, parasite prevalence, parasite richness

Introduction

Species invasions represent one of the greatest threats to worldwide biodiversity (Carlton and Geller 1993; Vitousek et al. 1997; Crowl et al. 2008); yet much remains to be understood regarding non-indigenous species (NIS), their populations, and the communities they invade, especially in marine systems (Torchin et al. 2002). Due to enhanced globalization, marine species are being transported to new areas at an increasing rate through human-mediated introduction vectors, such as shipping and aquaculture (Ruiz et al. 2000). While many anthropogenically-transported species do not become established

(Carlton 1996; Williamson and Fitter 1996), successfully invading species may achieve larger body sizes and higher abundances than conspecifics in their native range (Torchin et al. 2001; Mitchell and Power 2003). NIS may also displace native species, alter community structure, and potentially facilitate the success of other invasive species (Simberloff and von Holle 1999; Molnar et al. 2008; Olyarnik et al. 2009).

Given the inherent challenges associated with becoming established in a novel environment, the success of NIS can sometimes represent a puzzling phenomenon. One proposed explanation for that success is that NIS may leave behind natural enemies in their native ranges (i.e., enemy release)

and gain a competitive advantage over ecologically similar species that are more hindered by predators, competitors, and parasites (Keane and Crawley 2002; Torchin and Mitchell 2004; Liu and Stiling 2006; Torchin and Lafferty 2009). In fact, NIS across numerous taxa have been documented with fewer parasite species in their introduced versus native ranges (Torchin et al. 2003; Torchin and Lafferty 2009; Jeschke et al. 2012). In marine systems, parasite richness in introduced species is often half that of native conspecifics (Torchin et al. 2002; Blakeslee et al. 2013). Moreover, in some cases, parasite escape has been associated with increased demographic performance in non-native hosts (Torchin et al. 2001; Blakeslee et al. 2013).

The degree to which NIS may escape parasites often depends on the vector of introduction, the distance between native and non-native regions, parasite life cycle complexity, and the time since introduction (Prenter et al. 2004; Torchin and Mitchell 2004; Blakeslee et al. 2013). For example, many marine NIS are transported as larvae in the ballast water of ships (Carlton and Geller 1993; Leppäkoski et al. 2002; Davidson and Simkanin 2012; Frazier et al. 2013; David et al. 2015). However, because marine larval stages are typically unparasitized, few parasite species may accompany a NIS host introduced via this vector (Torchin and Lafferty 2009; Blakeslee et al. 2013), or they may have difficulties establishing due to low founding populations (Torchin et al. 2002; MacLeod et al. 2010). Several parasite taxa (e.g., digenean trematodes) also require multiple hosts to complete life cycles and may utilize three or more species under varying degrees of host specificity (Lauckner 1980; Combes et al. 1994; Curtis 2003), further influencing the successful establishment of invading parasites. Even so, parasite diversity in an invasive host could increase (and parasite escape decrease) with time if more parasites become introduced from a host's native range (due to multiple introduction events), or if hosts acquire generalist parasites in their recipient region (Poulin and Mouillot 2003; Torchin and Mitchell 2004; Innocenti and Galil 2007).

Understanding the role that parasites may have in species invasions also requires an understanding of community composition and interspecific interactions in an NIS host's new region; i.e., native predators and competitors with which NIS may interact (Torchin and Mitchell 2004; Hatcher et al. 2006). This is because for NIS to have a competitive advantage over ecologically-similar native species, their native competitors must be negatively affected by parasites to a greater extent (Torchin and Lafferty 2009). While it is often assumed that a significant loss of

parasites in NIS will inherently provide a competitive edge to the invader, explicit evidence supporting that assumption is not always available or clear (Torchin et al. 2003; Colautti et al. 2004; Roche et al. 2010; Gendron et al. 2012). Moreover, even a baseline understanding of the types and abundances of parasites in native species may be poorly documented or missing for some species.

In our study, we focused on intertidal estuarine and coastal communities along the Atlantic coast of the United States, where a fairly recent invasive species, the Asian shore crab *Hemigrapsus sanguineus* De Haan, 1835, has become abundant and now overlaps with a group of native mud crabs (Panopaeidae) in many estuarine habitats (particularly those including rocks, cobble, or rubble) in the region. *Hemigrapsus sanguineus* is native to Asia, where it ranges from southern Russia to Hong Kong; it was first detected in eastern North America in 1988 in southern New Jersey and has since spread northwards to mid-coast Maine and southwards to North Carolina (Williams and McDermott 1990; McDermott 1991; McDermott 1998; Stephenson et al. 2009: Epifanio 2013). *Hemigrapsus sanguineus* is also introduced in Europe (since the late 1990s), where populations occur from Germany to France and recently also the UK (Schubart 2003; Micu et al. 2010; Dauvin and Dufossé 2011; Epifanio 2013; Seeley et al. 2015). Nine parasite species have been documented in *H. sanguineus* in its native range, including several trematode and rhizocephalan species (Blakeslee et al. 2009; McDermott 2011; Shields et al. 2015), while in its introduced North American range, only two parasites have previously been recorded (Shields et al. 2015): a larval nematode (Torchin et al. 2001; Blakeslee et al. 2009) and an acanthocephalan, *Profilicollis botulus* (Van Cleave, 1916) (Christiansen et al. 2009; Garcia-Varela et al. 2013).

Native panopeid mud crabs include several species, but two species are relatively common in moderate to higher salinity sites of eastern North America where *H. sanguineus* may also be found: the flatback mud crab *Eurypanopeus depressus* Smith, 1869 and the black-fingered mud crab *Panopeus herbstii* Milne-Edwards, 1834. Prior studies in Long Island Sound and southern New England over the past decade have documented increasing numbers of *H. sanguineus* concurrent with a decline in native mud crab abundance (Kraemer et al. 2007; O'Connor 2014). However, to date, a basic survey of the composition, richness, prevalence, and intensity of parasites in these mud crabs is lacking, yet needed to resolve any role parasites could play in their demographic performance or interactions in the region. One fairly conspicuous parasite species that has in

fact been well documented in panopeid mud crabs is a rhizocephalan barnacle, *Loxothylacus panopaei* (Gizzler, 1884). This parasite is native to the Gulf of Mexico, the Caribbean, Venezuela, and parts of Florida (Hines et al. 1997) but non-native throughout much of the southeastern and mid-Atlantic coastline of the US (Hines et al. 1997; Kruse and Hare 2007) with a recent invasion into Long Island Sound (Freeman et al. 2013). *Loxothylacus panopaei* has a unique life cycle among barnacles: it hijacks its host's internal physiology, extracts resources, changes its behavior, and ultimately castrates it, thereby eliminating individual fitness (Walker et al. 1992; Alvarez et al. 1995; Lafferty and Kuris 1999). Parasitized crabs also show higher mortality rates, a likely consequence of the nutrient diversion of the parasite (Alvarez et al. 1995). Thus this physiologically-damaging parasite can have a significant impact on host populations, especially when highly prevalent (Andrews 1980; Eash-Loucks et al. 2014). Yet the diversity and potential impact that other metazoan endoparasites may have on mud crabs in the region remain largely unknown.

In this study, we examined endoparasite taxonomic richness, prevalence, and intensity of infection in two species of native mud crab, *P. herbstii* and *E. depressus*, and the introduced Asian shore crab, *H. sanguineus*, from multiple estuarine and coastal communities along the US Atlantic coast. We also used previously published data and our own surveys to explore *H. sanguineus'* parasite escape through time. Finally, we assessed a reproductive body index (gonadosomatic index) to look for potential differences in reproductive allocation among crab species and by infection status.

Methods

Field sampling

Adult crabs (n=360) were collected at 11 estuarine and coastal sites (1 in Maine; 2 in Massachusetts; 7 on Long Island, New York; and one in New Jersey) during June–August 2014. Four of these sites (Garvies Point, Goldsmith, Tappen Beach, and Martha's Vineyard) were resampled in 2015 and one new site (Southampton) was added (Figure 1, supplementary material Table S1). At the majority of sites, crabs were haphazardly collected in the intertidal zone by looking under rocks, cobble, or searching through mud. At four sites on Long Island (Garvies Point, Jamaica Bay, Cold Spring Harbor, and Bay County Park), crabs were also collected from small milk crates full of autoclaved oyster shells that had been deployed in the lower intertidal zone for 2 months

(June through August), a collection methodology developed and successfully employed for over a decade at the Smithsonian Environmental Research Center (Fowler and Noble 2012). Our goal was to obtain at least 15–20 crabs per population. In some cases, fewer crabs were collected at sites with lower crab densities, qualitatively assessed based on sampling effort. This was especially true for mud crabs, where we anecdotally noted that sites with high densities of *H. sanguineus* tended to have lower densities of panopeids, or an absence of panopeids, as has been reported in prior quantitative field studies (Kraemer et al. 2007; O'Connor 2014). At one site (Cold Spring Harbor), we collected more mud crabs than other sites because we found a notably high abundance of the parasitic barnacle, *L. panopaei*. While we searched for all three species of crabs at each sample site, not all three were always found during our sampling. Moreover, because crabs were sometimes difficult to find, the size range of collected crabs at a site could be variable, especially for mud crabs (see Table 1). Moreover, sex ratios were significantly male skewed for *H. sanguineus* (79% male; $X^2=80.91$; df=1; p<0.0001) and *P. herbstii* (74% male; $X^2=21.73$; df=1; p<0.0001) but not for *E. depressus* (53% male; $X^2=0.83$; df=1; p=0.6615) (Figure 1, inset). As a result, more males were evaluated than females for many of the analyses described below. Following collection, crabs were frozen until processed in the lab.

Crab dissections and gonadosomatic index preparation and calculation

Prior to dissection, sex, carapace width (CW, measured as the widest point (mm) across the upper carapace using digital calipers), and missing limbs of each individual were noted. Only individuals with a carapace width greater than 8 mm were examined for endoparasites given the limited amount of internal tissue in very small crabs. Crabs were dissected by separating the upper from the lower carapace using a sterile razor blade, and all tissues from the hepatopancreas, gonad, and thoracic ganglia were transferred to glass slides and examined under a compound microscope to look for and count parasites, which were included as an infection intensity measure for each parasite taxon in Table 1. At Cold Spring Harbor, several mud crabs were found infected with *L. panopaei*, which was determined based on the presence of virgin or mature externae (reproductive sac of the parasite); however, these identifications represent a conservative estimate of infection given that some crabs may have been infected but not exhibiting externae.

Figure 1. Map of the US Atlantic coast showing sampling sites (black circles) of *Hemigrapsus sanguineus* (HS), *Eurypanopeus depressus* (ED), and *Panopeus herbstii* (PH). Numbers represent site names as described in supplementary material Table S1. The inset figure represents counts of individual crabs per sex and per species across all sample sites. Much of our sampling concentrated around Long Island because it is within the middle part of the species' ranges and not far from the initial record of introduction for *H. sanguineus*; also because the parasitic castrator *L. panopaei* recently invaded the area; and finally because it was nearby our base of operations, allowing for more in-depth coverage.
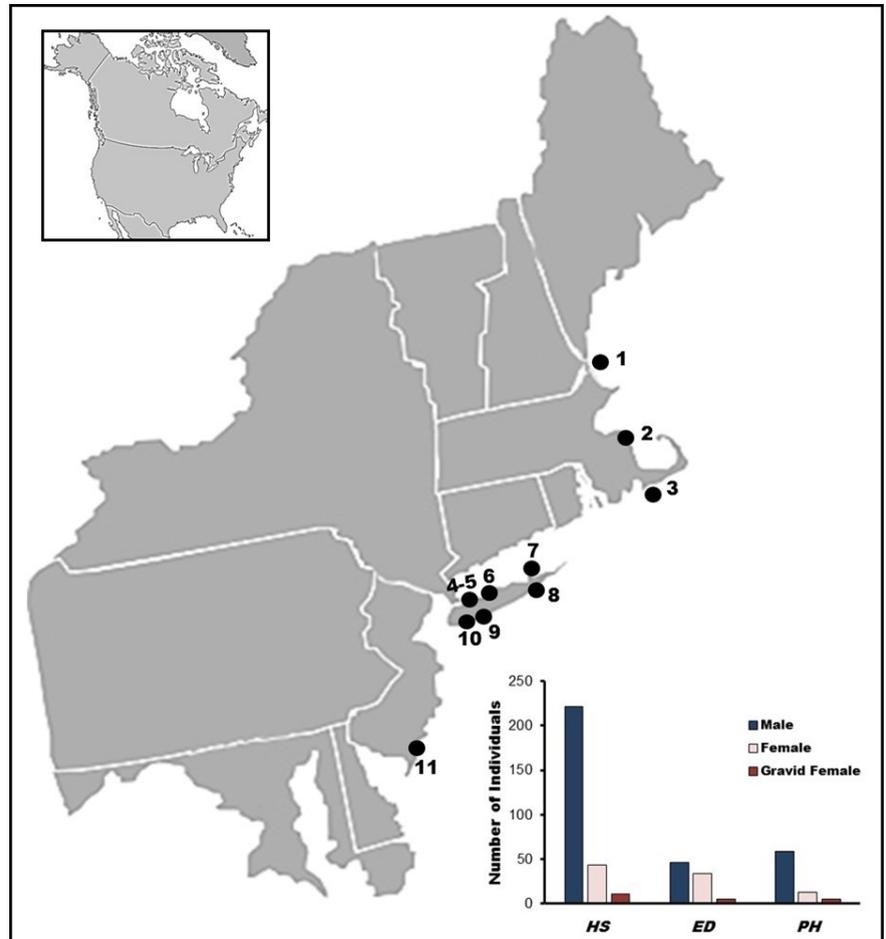
Moreover, since we just recorded presence or absence of *L. panopaei* based on visual observation of externae, we do not have an infection intensity measure for the rhizocephalan taxa. For the majority of the males and females sampled in 2014 (n=206), we calculated a gonadosomatic index (GSI) per crab, which is the ratio of dried gonad to the overall dried body weight of a crab (Griffen et al. 2011). To obtain a GSI value for each analyzed crab, all the gonad tissue within an individual was extracted and placed into an aluminum weigh boat; the rest of the body (including the carapace, claws, legs, and all other internal tissues) was placed into a separate aluminum weigh boat, and each sample was dried for 72 hours at 70 °C. The dried gonad and body were then weighed using an analytical balance. For those crabs missing walking legs or chelipeds, we accounted for missing limbs by obtaining dry weights (g) of a subset (n=28) of crab limbs and then regressing limb weights with crab carapace width to obtain a regression formula (*H. sanguineus* walking

legs: R^2=0.408; cheliped: R^2=0.8326; *E. depressus* walking legs: R^2=0.509; cheliped: R^2=0.3643) that we used to replace the weight of lost limbs depending on the crab's size. This was done because missing limbs can skew GSI estimates by reducing overall body mass used to calculate the index. Individuals with insufficient gonad tissue were not included in the analysis. Altogether, 102 male and 20 female *H. sanguineus*, 41 male and 11 female *P. herbstii*, and 28 male and 4 female *E. depressus* were evaluated for GSI.

Excystment and morphological analysis of a microphallid trematode in H. sanguineus

During our investigation, *H. sanguineus* was found infected by a previously unidentified microphallid trematode at one of our study sites (Tappen Beach, Sea Cliff, NY) (note: this trematode was initially disco-vered in *H. sanguineus* by C. Keogh and O. Miura during an unrelated parasite investigation; we then

Table 1. Taxa Richness (Rich), Absolute (Abs) Prevalence (Prev), and Taxa-Specific Prevalence and Intensities (Int) across sites for *Hemigrapsus sanguineus* (HS), *Eurypanopeus depressus* (ED), and *Panopeus herbstii* (PH). Also reported are the sample site (with site # on Figure 1), the sample year, the sample size, and the average (+SE) carapace width (size) of crabs. There are no intensities listed for rhizocephalans because we only noted presence or absence of infection for this parasite. For ED at Cold Spring Harbor, 47 crabs were assessed for the rhizocephalan parasite (*Loxothylacus panopaei*) and of those 47 crabs, 29 crabs were also assessed for other endoparasites. Trm=Trematoda; Acn=Acanthocephala; Nem=Nematoda; Rhz=Rhizocephala.

Crab Abbr	Site (#)	Year	Sample Size	Avg (±SE) size of crab	Taxa Rich	Abs Prev	Trm Prev	Trm Int	Acn Prev	Acn Int	Nem Prev	Nem Int	Rhz Prev
HS	Appledore Island, ME (1)	2014	19	17.51 (±0.69)	1	0.05	0.00	0	0.05	1	0.00	0	0.00
HS	Scituate Harbor, MA (2)	2014	20	18.00 (±0.74)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Martha's Vineyard, MA (3)	2014	20	20.62 (±0.89)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Garvies Point, Glen Cove, NY (4)	2014	25	26.83 (±1.04)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Garvies Point, Glen Cove, NY (4)	2015	20	21.04 (±0.61)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Tappen Beach, Sea Cliff, NY (5)	2014	20	19.53 (±0.91)	2	0.35	0.30	9	0.00	0	0.05	1	0.00
HS	Tappen Beach, Sea Cliff, NY (5)	2015	23	23.26 (±0.64)	1	0.48	0.48	26	0.00	0	0.00	0	0.00
HS	Goldsmith Inlet, Southold, NY (7)	2014	15	15.72 (±0.65)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Goldsmith Inlet, Southold, NY (7)	2015	18	20.24 (±0.57)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Southampton, NY (8)	2015	8	21.16 (±0.97)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
HS	Somers Point, NJ (11)	2014	20	18.74 (±1.02)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
ED	Garvies Point, Glen Cove, NY (4)	2014	8	21.43 (±2.80)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
ED	Garvies Point, Glen Cove, NY (4)	2015	3	15.62 (±2.31)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
ED	Cold Spring Harbor, NY (6)	2014	47 (29)	12.52 (±0.55)	1	0.64	0.00	0	0.00	0	0.00	0	0.64
ED	Bay County Park, NY (9)	2014	8	21.96 (±4.58)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
ED	Jamaica Bay, NY (10)	2014	16	19.74 (±0.72)	1	0.06	0.00	0	0.06	1	0.00	0	0.00
PH	Martha's Vineyard, MA (3)	2014	10	13.10 (±1.17)	1	0.30	0.30	5	0.00	0	0.00	0	0.00
PH	Martha's Vineyard, MA (3)	2015	6	11.63 (±1.05)	1	0.17	0.17	1	0.00	0	0.00	0	0.00
PH	Garvies Point, Glen Cove, NY (4)	2014	15	17.74 (±1.23)	2	0.14	0.00	2	0.07	1	0.07	1	0.00
PH	Goldsmith Inlet, Southold, NY (4)	2014	4	17.93 (±0.66)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
PH	Cold Spring Harbor, NY (6)	2014	5	13.33 (±2.36)	1	0.40	0.00	0	0.00	0	0.00	0	0.40
PH	Bay County Park, NY (9)	2014	12	21.24 (±2.18)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
PH	Jamaica Bay, NY (10)	2014	14	22.37 (±2.31)	0	0.00	0.00	0	0.00	0	0.00	0	0.00
PH	Somers Point, NJ (11)	2014	4	11.93 (±0.73)	0	0.00	0.00	0	0.00	0	0.00	0	0.00

carried out detailed surveys in the region to document its prevalence and extent). Because trematodes at the metacercarial stage can be difficult to morphologically identify, we induced excystment in several individuals to examine morphological characters of excysted worms.

Ten *H. sanguineus* crabs were anaesthetized by freezing at 0 °C for approximately 15–20 minutes. The crabs were then dissected and observed under a stereomicroscope for the presence of metacercarial cysts. These cysts were mostly found in the ganglia region and surrounding tissues within the crab's

body cavity. Once located, cysts were removed from the crab's tissue and placed into separate wells of a 96-well plate. These wells were randomly assigned either a trypsin (0.25%) or PBS saline buffer (pH 7.5) treatment. 300 µl of either trypsin or saline buffer were added to their assigned wells, and the plate was then incubated at 42 °C for 12 hours (following methods in Dunn et al. 1989). The plate was checked every 30 minutes for the first two hours under the stereomicroscope for excysted worms; after 12 hours, the trials were terminated. Images were captured of excysted worms and unexcysted metacercariae using a stereomicroscope and a compound microscope. After excystment, worms were examined for morphological characters to identify the microphallid species (Hunter 1952; Dunn et al. 1989).

Statistical analyses

Taxonomic richness was calculated as the number of parasite taxa per site per crab species. However, because varying numbers of crabs were analyzed per site, we adjusted site-level richness using rarefaction techniques in EstimateS 9.1.0 (Colwell et al. 2012) to predict the number of parasite taxa at a particular sampling effort and thus standardize across samples (as in Blakeslee and Byers 2008). We set that sampling effort at 5 individuals, which was the lowest sample size where parasites were detected (Table 1). We then tested for differences in richness among crabs using an ANOVA with adjusted taxa richness as the response variable and crab species as a categorical factor and site as a random effect. In addition, we constructed rarefaction curves (accumulated richness based on observed data and predicted richness based on an estimator) for each crab species across all sample sites using the output from EstimateS 9.1.0 (Colwell et al. 2012). Based on recommended use with parasite data (Walther and Morand 1998), we chose the nonparametric richness estimator, Chao2. Chao2 uses the frequency of unique taxa in samples (i.e., incidence-based) to estimate missing taxa in a population (Chao 2005). For each accumulation curve, we also included the extrapolation option in EstimateS to graphically demonstrate the predicted asymptote and the number of sampled infected crabs needed to attain the predicted parasite richness.

Absolute infection prevalence per site per crab species was determined as n_i/n, where n_i = the number of individuals infected with any parasite and n = the total number of individuals at a site. Both absolute prevalence and taxa-specific prevalence were calculated. Given the patchiness of parasitism across sites for many species, we transformed

prevalence using an anscombe arcsine square-root transformation (as advised by Zar 1999 for proportion-based statistical analyses; Altman and Byers 2014); this transformation returns positive non-zero values, using sample size, the number of infections, and the specific transformation calculations (see Zar 1999). Only sample sizes of 5 and higher were included in transformed prevalence analyses. To determine whether overall prevalence across parasite taxa differed among crab species, we ran an ANOVA with transformed prevalence as the response variable and crab species as a categorical factor with site as a random effect. We additionally investigated a multivariate analysis of taxa-specific prevalence using a MANOVA with transformed prevalence of our four parasite taxa as response variables and crab species as a categorical factor with site as a random effect.

GSI was calculated as the ratio of dried gonad to dried body mass after adjusting for missing limbs (as described above). To determine whether GSI differed among crab species, sexes, and infection status, an ANOVA was performed with GSI as the response variable and crab species, sex, and infection status (Y/N) as categorical factors, and site as a random effect.

Finally, parasite escape in *H. sanguineus* was calculated using the formula (N-I)/N, where N = the total parasite richness of the native range (9 species) and I = the total parasite richness of the introduced Atlantic coast range (Torchin et al. 2001, 2003; Blakeslee et al. 2009). Total parasite richness of the introduced range and calculations for parasite escape for five time points (2001, 2009, 2011, 2013, and 2015) included parasite data from this study and prior studies (Torchin et al. 2001; Blakeslee et al. 2009; Christiansen et al. 2009; McDermott 2011; Blakeslee et al. 2013). Time points were defined as the publication year of each study because the exact dates of parasite discoveries in US populations were not always clear. A linear regression of parasite escape and time were then analyzed to determine if parasite escape had declined with time. All statistical analyses were performed using JMP 12.0.1 (SAS Institute, Inc.).

Results

Parasite taxa and identifications

During our investigation, three parasite taxa were found infecting *Hemigrapsus sanguineus*: a microphallid trematode, a nematode, and an acanthocephalan. This latter parasite may be the same acanthocephalan species *Profilicollis botulus* (Van Cleave, 1916) that
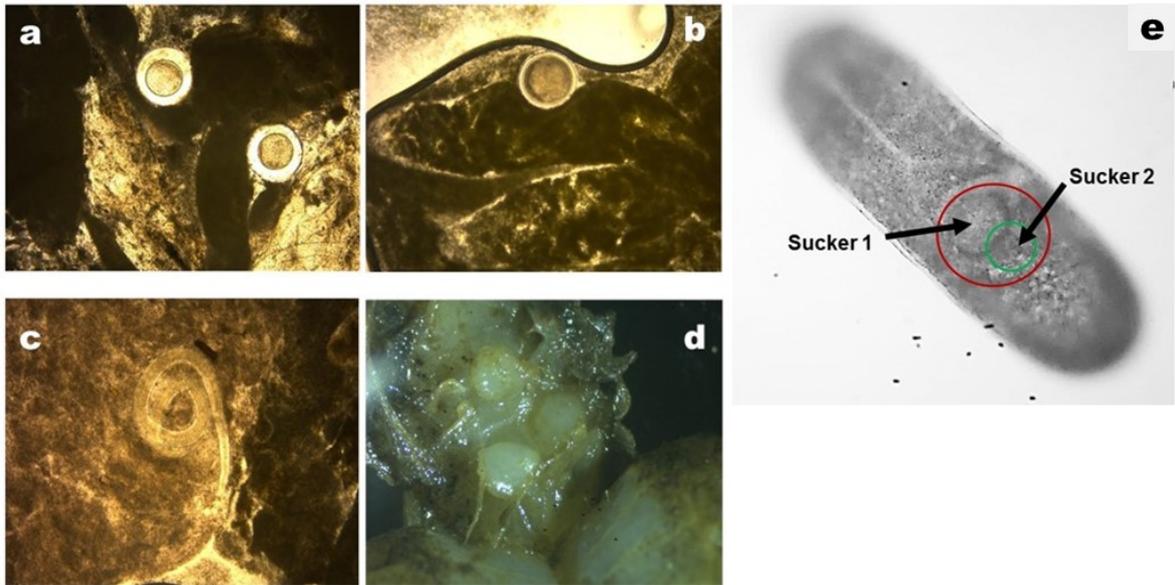
Figure 2. Parasites observed in *Hemigrapsus sanguineus*, *Eurypanopeus depressus*, and *Panopeus herbstii* crabs. (a) Trematode metacercariae in *P. herbstii* from Martha's Vineyard, MA; (b) Trematode metacercaria in *H. sanguineus* from Tappen Beach Park, Sea Cliff, NY; (c) Nematode in *P. herbstii* from Garvies Point, NY; (d) Virgin externa of the parasite *Loxothylacus panopaei* in *E. depressus* from Cold Spring Harbor, N; and e) Excysted *Gynaecotyla adunca* from an infected *H. sanguineus* crab following a 0.25% trypsin treatment—the two ventral suckers are circled. Photomicrographs by KL Kroft and AMH Blakeslee.

was observed by Christiansen et al. (2009) in *H. sanguineus* and in another non-native crab in the region, the European green crab *Carcinus maenas* (Linnaeus, 1758) (Torchin et al. 2001; Blakeslee et al. 2009). Four parasite taxa infected mud crabs: a microphallid trematode, the rhizocephalan *Loxothylacus panopaei* (Gissler, 1884), a nematode, and an acantho-cephalan (Figure 2a-d). This acanthocephalan may also be the same species (*P. botulus*) observed in these other intertidal crabs, but we were unable to confirm this. The rhizocephalan *L. panopaei* was only observed at one of our sample sites (Cold Spring Harbor, NY), mostly infecting *E. depressus*, where infection prevalence was 64%.

The discovery of a microphallid trematode infecting *H. sanguineus* at the Tappen Beach (Hempstead Harbor, Long Island) site represents a new parasite record (and the first trematode record) for *H. sanguineus* in its non-native range of eastern North America. However, we did not see this trematode at any other site where we sampled *H. sanguineus*. Based on metacercarial and excysted worm morpho-logies, we believe this microphallid is a native North American species *Gynaecotyla adunca* (Linton, 1905) that infects the abundant eastern mudsnail *Ilyanassa obsoleta* (Say, 1822) as first-intermediate host and several crustacean species as second intermediate host (Curtis 2003; Blakeslee et al. 2012).

During excystment trials, all extracted metacercariae excysted in the trypsin but not the saline buffer treatments as in Dunn et al. (1989), and the morphology of the excysted worm was most similar to that described in Hunter (1952) for adult worms of *G. adunca*, including the presence of two ventral suckers (Hunter 1952; Pung, pers. comm.; Figure 2e). We were unable to carry out a similar excystment trial on the microphallid trematode we found infecting *P. herbstii* due to low sample size of cysts; therefore, the microphallid species infecting this crab remains unknown.

Taxonomic richness, prevalence, infection intensity, and species rarefaction

Adjusted taxonomic richness was not significantly different among the three species of crab ($F_{2,14}=2.0915$; p=0.1601; Table 1, Figure 3a). Transformed prevalence was found to significantly differ among the crabs ($F_{2,10}=4.9879$; p=0.0304), and in a post-hoc Tukey's test, *H. sanguineus* had significantly lower prevalence (p<0.05) than *P. herbstii,* but not *E. depressus* (Figure 3b); however, no significant differences were found among the crab species in a MANOVA of transformed prevalence for our four parasite taxa (Wilks' Lambda $F_{8,30}=1.8928$; p=0.0984). Infection intensities for the trematode,
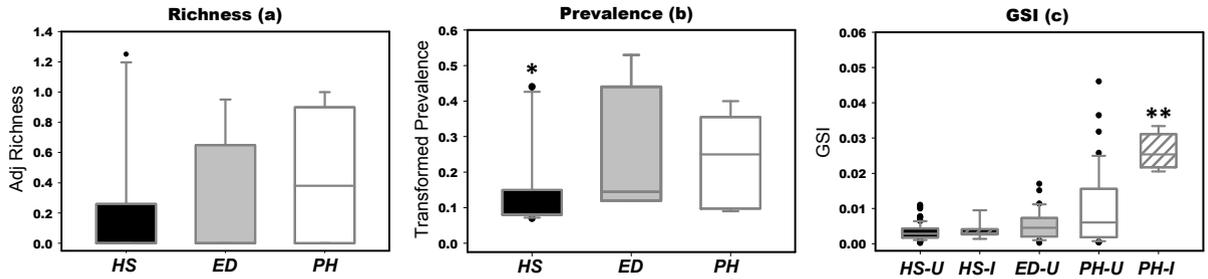
Figure 3. Box plots of adjusted (adj) parasite richness, transformed prevalence, and GSI (uninfected (U) or infected (I)) for the three crab species. This figure demonstrates three parasite analyses of *H. sanguineus* (HS), *E. depressus* (ED), and *P. herbstii* (PH) across our study (with site included as a random effect): (a) Adjusted parasite taxonomic richness; (b) Transformed infection prevalence; and (c) GSI for uninfected and infected crabs. *represents a significant effect of crab species on infection prevalence, with HS having significantly lower prevalence than PH. **represents a significant interaction for crab and infection status, with infected PH having a significantly higher GSI than all other comparisons (uninfected HS, infected HS, uninfected ED, and uninfected PH).
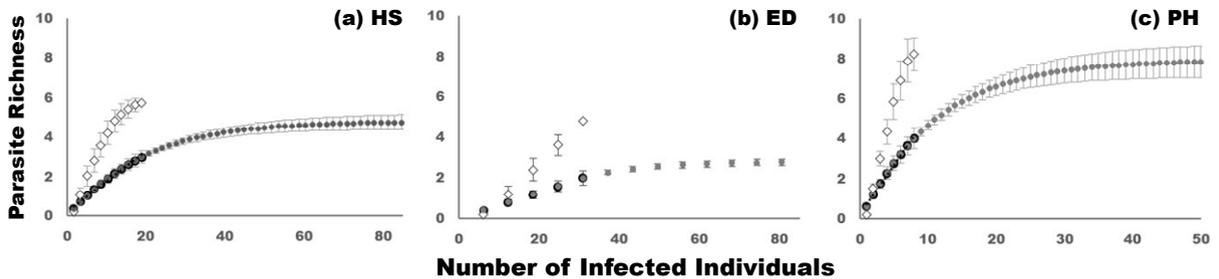
Figure 4. Rarefaction curves of parasite richness data for (a) *Hemigrapsus sanguineus* (HS), (b) *Eurypanopeus depressus* (ED), and (c) *Panopeus herbstii* (PH). Curves represent the accumulated number of observed parasite species (black circles), including extrapolation to the asymptote (gray circles), and estimated number of species (open triangles) across the number of infected individuals for each crab species. The Chao2 estimator was used to estimate parasite richness. Error bars are standard error.

nematode, and acanthocephalan taxa were fairly low across all sites, except for trematode infection at Tappen Beach Park (Sea Cliff, NY) in parasitized *H. sanguineus* (Table 1).

In rarefaction analyses, *H. sanguineus* observed (3.00±0.32) and estimated (5.72±0.01) parasite richness for 19 infected individuals was slightly higher than *E. depressus* observed (2.00±0.34) and estimated (4.80±0.01) parasite richness for 31 infected individuals, while *P. herbstii* observed (4.00±0.50) and estimated (8.23±0.82) species richness for 8 infected individuals was slightly higher than both *H. sanguineus* and *E. depressus*. Extrapolation analyses for both *H. sanguineus* and *E. depressus* suggested that approximately 80 infected crabs of each species would need to be sampled to reach predicted parasite richness in the sampling region, while for *P. herbstii* that number was approximately 50 infected crabs (Figure 4). These rarefaction analyses suggest that further sampling could reveal more parasite species for each crab species.

Gonadosomatic index

The average GSI (±SE) across sites was 0.0039±0.0006 (n=117) for *H. sanguineus*, 0.0054±0.0007 for *E. depressus* (n=31)*,* and 0.0119±0.0015 for *P. herbstii* (n=52). When exploring GSI by infection status for each crab, the average GSI for infected *H. sanguineus* was 0.0038±0.0009 (n=8) and was 0.0039±0.0007 (n=109) for uninfected *H. sanguineus*. For *P. herbstii*, infected GSI was 0.0262±0.0051 (n=5) and uninfected GSI was 0.0103±0.0015 (n=47). For *E. depressus,* there were not enough infected individuals with GSI data to compare infected versus uninfected (most *E. depressus* infections were by the rhizocephalan, *L. panopaei*, in small crabs with little or no detectable gonad tissue).

The effects of crab species ($F_{2,156}$=22.3103; p<0.0001) and infection status ($F_{2,200}$=12.6853; p=0.0005) were statistically significant, while sex ($F_{1,200}$=2.2413; p=0.1359) was not. In a post-hoc Tukey's test, *P. herbstii* had a significantly (p<0.05) higher GSI than *H. sanguineus* but neither were

significantly different than *E. depressus*, and in a student's t-test, infected crabs had a significantly (p<0.05) higher GSI than uninfected crabs. There was a significant interaction between crab and infection status ($F_{2,182}$=8.2484; p=0.0004) and in a post-hoc Tukey's test, infected *P. herbstii* had a significantly (p<0.05) higher GSI than all other comparisons (i.e., uninfected *P. herbstii*, both infected and uninfected *H. sanguineus*, and uninfected *E. depressus*) (Figure 3c).
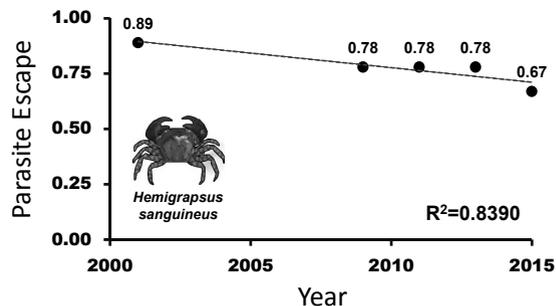
Parasite escape in Hemigrapsus sanguineus

In 2001, parasite escape was calculated to be 0.89 (9 parasites in the native range versus 1 parasite, a nematode, in the introduced range; Torchin et al. 2001). In 2009, it declined to 0.78 with the discovery of the acanthocephalan (thus 9 versus 2) (Christiansen et al. 2009). In 2011 and 2013, parasite escape continued to be 0.78 (McDermott 2011; Blakeslee et al. 2013). Finally, in 2015, we detected a third parasite species (microphallid trematode) in the non-native range, reducing parasite escape to 0.67. Altogether, this represented a significant negative relationship when regressed with time (R^2=0.839; n=5; p=0.029) (Figure 5). However, because the nematode initially discovered by Torchin et al. (2001) was unindentified, as were records of a nematode in later studies (e.g., Blakeslee et al. 2009), we cannot be certain whether this latter nematode represents the same or a different species. As such, we reanalyzed parasite escape with time to reflect the potential for two nematode species from 2009 onwards (i.e., parasite escape in 2001 = 0.89, from 2009–2013 = 0.67, and in 2015 = 0.56). This reanalysis revealed a stronger relationship of parasite escape with time (R^2=0.927; n=5; p=0.009).

Discussion

Parasite escape and novel parasite acquisition in Hemigrapsus sanguineus

In eastern North America, *Hemigrapsus sanguineus* appears to have left behind all the known parasites of its native range, and though it has acquired new parasites (McDermott 2011), it continues to be infected by one-third the parasite taxa infecting conspecifics in native Asia. Yet the crab's parasite escape has declined over time: in 2001, only one parasite (a larval nematode) was detected in non-native populations (Torchin et al. 2001), and by 2015, there were three (a nematode, an acanthocephalan, and a trematode). Even so, the distribution and abundance of infected *H. sanguineus* in eastern North America

Figure 5. Regression of parasite escape and time for *Hemigrapsus sanguineus*. Parasite escape was regressed with five time points to determine if it would show a linear decline with time given the possibility for multiple introductions and accumulation of native parasites. Numbers above the data points represent parasite escape values for each time point. Parasite escape showed a significant linear decline with time (p=0.0290).

was fairly limited throughout our sampling range: the nematode was found at just one site (prevalence=0.05); the acanthocephalan at one site (prevalence=0.05); and the trematode at one site (prevalence=0.30 in 2014 and 0.48 in 2015). Moreover, infection intensities for these parasite taxa were low, except for the trematode, in which infection intensity was actually higher than either of the two mud crab species (note: we cannot compare to rhizocephalan infection intensity in mud crabs since we only collected presence/absence data for this taxon). Moreover, rarefaction analyses suggested that a couple additional parasite species could be found in *H. sanguineus* with more sampling, and in fact, the decline in parasite escape we observed over time could certainly be influenced by a greater sampling effort for parasites in *H. sanguineus* over time at more sites, enhancing the likelihood that new parasites could be detected.

Yet, declines in parasite escape over time have also been observed for other aquatic NIS, indicating that it may be common for NIS to acquire parasites in the non-native range or for individuals to transport parasites from the host range during subsequent introduction events (Torchin and Mitchell 2004). For example, Torchin and Lafferty (2009) demonstrated that older introduced populations of the European green crab have higher numbers of parasite species than more recently introduced populations. In addition, Gendron et al. (2012) found that introduced populations of the Eurasian round goby in the Great Lakes have more than twice the number of parasite taxa than they did 15 years earlier. Of interest is that *H. sanguineus* has shown a relatively rapid increase in parasite richness over time: it invaded eastern North America about 25 years ago and is presently

infected by three parasite taxa. In comparison, *C. maenas* invaded eastern North America approximately 200 years ago and is also infected by three parasite taxa (Torchin et al. 2001; Blakeslee et al. 2009), which happen to be the same taxa as *H. sanguineus*: a nematode, an acanthocephalan, and a trematode (Blakeslee et al. 2009). This may indicate that some parasite taxa are more readily acquired upon introduction or transferred with their hosts (Torchin and Mitchell 2004; Blakeslee et al. 2013), and also that a significant release from parasites could be maintained for a substantial period of time; i.e., *C. maenas* has one-third its native parasite load 200 years after introduction (Blakeslee et al. 2009). However, without historical data, it is impossible to ascertain how long it took for *C. maenas* to acquire its current parasite load; it also remains unclear whether the trematode parasite (*Microphallus similis*) that infects it in eastern North America was transported from Europe, acquired from eastern North America post-introduction, or a combination of both (Blakeslee et al. 2015). Interestingly, *H. sanguineus* has also been introduced to Europe, but parasite composition and richness are yet to be investigated in this region (McDermott 2011), along with how parasites could influence interactions between *H. sanguineus* and *C. maenas* in eastern North America where both are non-native, and in Europe, where *C. maenas* is native and *H. sanguineus* is introduced.

Though *H. sanguineus* has clearly escaped parasites in eastern North America, it is also important to note that parasite escape might be overstated if an entire native range is used for its calculation rather than the specific source region from which the introduction originated (Colautti et al. 2004; Colautti et al. 2006). A recent analysis of this potential issue found little evidence of a "source effect" for parasite escape when averaged across 6 marine invertebrate host species for which a more precise source region was known (Blakeslee 2016). For *H. sanguineus,* specifically, the source area for the US Atlantic introduction appears to be Japan (based on genetic analysis; A. Blakeslee, O. Miura, unpublished data), and the majority of the field records used to construct parasite escape in the native range have come from Japan (see Blakeslee et al. 2009). Thus a source effect may be less influential for our analysis here. Even so, many more studies are needed to truly investigate this question, especially because parasite records in the native range are often accumulated from publications spanning multiple years or decades, while the invasive range may only represent a handful of recent publications.

Here, we also report the first documentation of a microphallid trematode infection in *H. sanguineus* in its eastern North American range. Based on morphological analysis of excysted worms, it appears that this trematode is a native microphallid, *Gynaecotyla adunca*, which uses the eastern mudsnail *Ilyanassa obsoleta* as its first-intermediate host, crustaceans like fiddler crabs and amphipods as second-intermediate hosts, and shorebirds and marsh birds as definitive hosts (Hunter and Vernberg 1953; Heard 1970; Curtis 1987; Dunn et al. 1990). Such host switching between native trematodes and NIS has been documented in other coastal systems, including the North Sea, where two invasive mollusks, the Pacific oyster and the American razor clam, are now being used as hosts by trematodes that switched from native mollusks in the region (Krakau et al. 2006). Similar to *H. sanguineus*, these two mollusks are relatively recent invaders of the North Sea, suggesting that host switching can occur fairly rapidly. Moreover, host switching can have important impacts on invaded communities, especially for native hosts. If parasites begin utilizing invasive hosts, the parasite load on native species could be reduced (i.e., parasite sink), possibly providing a benefit for these species (Krakau et al. 2006), or alternatively, the addition of competent hosts in a region could increase parasite abundance and "spill back" to native hosts (Kelly et al. 2009). However, significant infection of the introduced species would need to occur for these possible consequences to be derived. At this point, it is unclear how much of an effect *H. sanguineus* infection could have in the region, especially given the limited distribution of parasites infecting the crab in its US range; e.g., we did not detect the trematode species (*G. adunca*) at any other site in either 2014 or 2015, even though we sampled a nearby (<2 km) site within the same harbor. Yet prevalence and intensity of trematode infection at this site was relatively high, and so continued monitoring at this and nearby sites would be valuable to determine any community-level effects of trematode parasitism in the region.

It is also unclear whether the parasites *H. sanguineus* has newly acquired could have individual or population-level influences in the non-native range. Trematode infections, in particular, have been shown to have a wide variety of effects on hosts. For instance, microphallid metacercariae are known to cause tissue damage, activate immune response, and lower growth rates, all of which could impact a host's fitness (Martorelli and Schuldt 1990; Robaldo et al. 1999; Thieltges 2006; Blakeslee et al. 2015). Moreover, trematode infection can elicit behavioral changes in hosts that increase parasite transmission to the next host (Lafferty 1999; Chubb et al. 2010). In fact, the microphallid trematode we found in

H. sanguineus (*G. adunca*) has been implicated in behavioral changes in its first intermediate host in that infected snails moved higher up the beach where they were in greater contact with upper intertidal second-intermediate hosts (Curtis 1987). Some of the *G. adunca* metacercariae observed in *H. sanguineus* were found in the crab's thoracic ganglia (i.e., nerve center), possibly enhancing the likelihood for any behavioral effects, and this possibility warrants future investigation.

Parasite infection in native mud crab species

Compared to *H. sanguineus*, average richness was not significantly different for either native mud crab species, and rarefaction curves were fairly similar among the crabs, though parasite richness in *P. herbstii* was predicted to include a few more species than *H. sanguineus* and *E. depressus*. In contrast, prevalence of parasite infection was significantly lower for *H. sanguineus* than *P. herbstii,* but not *E. depressus,* when taking into account all parasite species. However, when taxa-specific prevalence was assessed, crabs were not significantly different from one another, suggesting that parasite taxa can be a strong influence in this system. In particular, prevalence and intensity of infection in acantho-cephalans and nematodes were fairly rare across all three species, but in contrast both measures were relatively high for trematode infection at one site for *H. sanguineus*, and relatively high for rhizocephalan prevalence at one site for *E. depressus*. Several studies across terrestrial and aquatic habitats have found significant differences in parasite diversity between native and non-native hosts (e.g., Aliabadi and Juliano 2002; Torchin et al. 2005; Hatcher et al. 2006), which could influence competitive interactions between the native (more parasitized and presumably less healthy) and non-native (less parasitized and presumably more healthy) hosts. In our study, these kinds of baseline data were not previously available for native mud crab species and their invasive competitor, *H. sanguineus*, and a continued under-standing of these measures are important, especially given the possibility for further documentation of parasites in all three species.

As noted above, a parasite we found in high prevalence at one Long Island site (Cold Spring Harbor) was the invasive rhizocephalan barnacle, *L. panopaei.* Only small (CW < 15mm) mud crabs (primarily *E. depressus*) were infected, likely because small crabs molt frequently, making them more susceptible to infection since *L. panopaei* larvae are better able to penetrate the host cuticle (Hines et al. 1997; Shields et al. 2015). Moreover, this parasite prevents hosts from growing larger by arresting molting

(Alvarez et al. 1995). While we found *L. panopaei* infecting mud crabs at Cold Spring Harbor, field surveys at our other sample sites did not reveal additional *L. panopaei* populations. However, Freeman et al. (2013) noted a couple other northwestern Long Island sites with *L. panopaei* infections in *E. depressus*; thus further surveying is important in this system, especially because continued spread throughout the region could be detrimental to native mud crab populations in the Northeast USA (Freeman et al. 2013). Rhizocephalan parasites impair host growth, reproduction, and survival, and *L. panopaei*, in particular, has been shown to reach high infection rates in some invasive populations on the Atlantic coast of North America (Lafferty 1993; Alvarez et al. 1995; Hines et al. 1997; Kruse and Hare 2007; Kruse et al. 2012). In fact, in southern USA populations where *L. panopaei* is also invasive, *E. depressus* density declines associated with *L. panopaei* infection prevalence have been documented (Eash-Loucks et al. 2014). Unlike its mud crab competitors in eastern North America, *H. sanguineus* remains uninfected by parasitic castrators — providing it an escape from a particularly damaging parasite group. In its native Asian range, *H. sanguineus* is infected by two rhizocephalan species (Blakeslee et al. 2009), and lab studies of *H. sanguineus* infected with its native rhizocephalan parasite, *Sacculina senta* (Boschma, 1933), found the annual growth of parasitized individuals to be lower than healthy individuals (Takahashi and Matsuura 1994). Moreover, in a study on the invasive European green crab, *C. maenas*, Torchin et al. (2001) found that populations with lower prevalence of parasitic castrators had higher abundances and larger body sizes compared to native conspecifics. In fact, recent evidence in a South Carolina population of *E. depressus* infected by invasive *L. panopaei* has found reduced foraging efficiency in infected versus uninfected *E. depressus* crabs, which may have population- and community-level impacts given the influence on predator-prey interactions (Toscano et al. 2014). Therefore, the lack of parasitic castrators in introduced *H. sanguineus* populations may confer a fitness advantage compared to native mud crabs facing rhizocephalan infection. Further research is needed to determine whether rhizocephalan infection in mud crabs, and lack thereof in invasive *H. sanguineus,* could influence species interactions among these crab species.

GSI analyses and influence of infection status

In analyses of gonadosomatic index (GSI) across our study sites, *P. herbstii* was found to have a significantly higher GSI than either *H. sanguineus* or

E. depressus, suggesting that *P. herbstii* had the largest investment in its gonad tissues (during our sampling period) of the three crabs. Interestingly, in a study of female egg production by Hartnoll (2006), percent investment per brood, broods per year, and percent investment per year were similar or lower for *P. herbstii* compared to *E. depressus.* It is possible that our results differed from this prior work because of the strong skew towards males in *P. herbstii* in our study. *Hemigrapsus sanguineus* demonstrated a significantly lower GSI than *P. herbstii,* which may be due to species-specific differences in reproductive strategies between the crabs. For example, it has been suggested that *H. sanguineus* is an income breeder, taking in energy for reproduction as needed during the breeding season rather than storing energy as many other crab species do. Indeed, this strategy could be contributing to *H. sanguineus'* success as an invasive species since it allows for prolonged reproduction (Griffen et al. 2012). Additionally, it may be that *H. sanguineus* is allocating more energy towards physiological processes that allow it to tolerate a wide range of environments in its invasive range (Epifanio et al. 1998).

Notably, we also found GSI to be influenced by infection status: GSI was significantly higher in infected *P. herbstii.* This may suggest more investment in reproduction in infected versus uninfected *P. herbstii* crabs. Other studies have shown parasite infection can influence reproductive investment, presumably as a response to parasitism stress. For example, in a study of first-intermediate gastropod hosts infected with castrating trematodes, female snails increased egg production during initial stages of infection, and egg production declined as the infection progressed. Moreover, studies have also shown snails in high trematode prevalence populations to mature earlier than snails in lower prevalence populations (Minchella and Loverde 1981; Gérard and Théron 1997; Granovitch et al. 2009). For *P. herbstii,* parasitic infection could be inducing a similar type of stress response; i.e., enhancing reproductive investment in infected individuals. However, this result should be treated with caution due to the small sample size of infected *P. herbstii.*

Conclusions

Parasites play an important role in marine ecosystems and may affect the outcome of invasions and the impact of NIS on novel communities. In this study, *Hemigrapsus sanguineus* was found infected with a higher number of parasite species than observed in previous years on the US Atlantic coast. This finding

contributes to past evidence demonstrating that NIS can accumulate parasites over time, though populations may be parasite free or have low levels of infection initially following introduction. Depending on the extent to which parasites affect populations of NIS, further accumulation of parasites could influence performance over time. This can have important implications for the invaded community if host switching allows a parasite's native hosts to become less heavily parasitized as a result, or if an increase of competent hosts could spillback to native hosts increasing parasite abundance in a region. Furthermore, NIS may continue to have an advantage over native competitors if they are infected by fewer, significantly-costly parasites. For example, in some populations where *H. sanguineus* and mud crabs overlap, panopeid mud crabs can have high prevalence of infection by the castrating rhizocephalan, *L. panopaei,* which has been shown to elicit considerable impacts on host physiology and behavior. Future studies are needed to assess the effects of parasitism on fitness in introduced populations of *H. sanguineus* as well as the effects of parasitism on competitive interactions between *H. sanguineus* and mud crabs. For example, it would be informative to investigate whether mud crabs are less efficient competitors when infected by parasites compared to *H. sanguineus* (Toscano et al. 2014). Sampling more extensively in *H. sanguineus'* introduced range and over time are necessary to continue quantifying parasitism in this NIS and understand its ultimate effect on native communities with which it now interacts.

Acknowledgements

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Supplementary material

The following supplementary material is available for this article:

Table S1. Collection locations.

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