



# Reduced fecundity by one invader in the presence of another: A potential mechanism leading to species replacement

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## ARTICLE INFO

### Article history:

Received 21 December 2010

Received in revised form 27 May 2011

Accepted 1 June 2011

Available online xxx

### Keywords:

*Carcinus maenas*

Geographic variation

*Hemigrapsus sanguineus*

Invasive species

## ABSTRACT

As invasive species proliferate and expand their ranges, they often interact either with natives or with other invasives across a broad geographic range. Moreover, because geographic ranges span a diversity of environments, the outcome of interactions between species pairs may vary spatially. The European green crab *Carcinus maenas* and the Asian shore crab *Hemigrapsus sanguineus* are both introduced species in North America where they co-occur over a large portion of the Atlantic coast. While interactions between the two crabs in the southern portion of this range within Long Island Sound resulted in the elimination of *C. maenas* within 2–3 years of *H. sanguineus*' arrival, species replacement appears to be taking much longer in northern areas within the Gulf of Maine. Previous work implicates predation by *H. sanguineus* on *C. maenas* recruits as the mechanism underlying species replacement. Here we explore an alternative or additional mechanism underlying this species replacement that can also account for the observed spatial variation in the timescale of species replacement between northern and southern areas. Specifically, we demonstrate that a previously documented shift in *C. maenas* diet which occurs in the presence of *H. sanguineus* can cause a reduction in *C. maenas* fecundity. This, combined with near-shore current patterns may explain the regional differences in the outcome of this species interaction.

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## 1. Introduction

Invasive species are increasingly prevalent in all types of biological communities and often have devastating and far reaching impacts. As of 2004, 42% of all species listed as Threatened or Endangered within the United States were at risk primarily because of the influence of invasive species (Pimentel et al., 2005). As the number of invasions continues to mount, interactions increasingly occur not just between invasives and natives, but also between multiple invasive species (Simberloff and Von Holle, 1999). And, similar to the threat of replacement imposed by invaders upon native species, interactions with new invaders can also compromise the persistence of previously established invasive species.

Following the introduction of a non-native species in a single location, invaders establish and proliferate, expand their ranges, and by doing so interact with resident species over increasingly extensive geographic scales (e.g., Nowak et al., 2008; Zeidberg and Robison, 2007). Ecological interactions that span broad geographic ranges are often characterized by spatial variation that results from local differences in behavior (Foster and Endler, 1999), genetics (e.g.,

Sanford et al., 2003), or environmental conditions (e.g., Paine 1966, Dethier and Duggins 1988). Thus, interactions and impacts of broadly-dispersed invasive species assessed from only a single location may not capture the full scale or diversity of their importance/influence across their entire invaded range.

Here we examine the interactions between two invasive crabs that occur in rocky intertidal areas across a broad latitudinal range on the east coast of North America. The European green crab *Carcinus maenas* was first introduced to North America in the early 1800s around New Jersey (Say, 1817). Over the next 150 years, it gradually expanded its range and is currently distributed from Nova Scotia to Maryland. The presence and abundance of *C. maenas* in different regions across this range are the result of complex interactions governed by genetic constraints (Roman, 2006), reproductive ecology (Audet et al., 2008), predation from birds (Dumas and Witman, 1993) and blue crabs (deRivera et al., 2005), the availability of refuge habitat (Griffen et al., 2008) and food resources (Griffen, 2009), and aggressive interactions between conspecifics (Griffen and Delaney, 2007). Added to this long list are interactions with another recently introduced intertidal crab, the Asian shore crab *Hemigrapsus sanguineus*. First noted in New Jersey in 1988 (Williams and Mcdermott, 1990), *H. sanguineus* quickly spread to encompass its current range from central Maine to North Carolina (Delaney et al., 2008). While *C. maenas* is generally found in a broader array of habitat types, both species are common in rocky

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intertidal areas where there is extensive overlap in both habitat and food preferences (Lohrer et al., 2000; Tyrrell and Harris, 1999).

The area cohabited by these two species encompasses two well studied regions that differ both in physical characteristics and in the outcomes of this species interaction. Southern areas within Long Island Sound are characterized by relatively warm water temperatures, limited wave exposure due to the enclosed nature of the sound, relatively few rocky intertidal sites, and relatively high nitrogen levels (i.e., eutrophic). The arrival of *H. sanguineus* to this region resulted in the rapid disappearance of *C. maenas* from rocky intertidal sites within just ~3 years (Kraemer et al., 2007; Lohrer and Whitlatch, 2002). In contrast, northern areas within the Gulf of Maine are characterized by colder water temperatures, more intense wave action, more abundant rocky intertidal habitats that provide excellent and abundant refuge for both species, and less nitrogen input. *H. sanguineus* first expanded its range into this region in the 1990s (McDermott, 1998a), and since this time declines of *C. maenas* have been much slower than was observed further south in Long Island Sound resulting in the coexistence of these two invasive species at the same sites within the Gulf of Maine for more than 10 years. Outcomes of this interspecific interaction in northern and southern regions may stem from differences in habitat and/or other environmental factors that vary between these two regions (Vermeij, 1978).

Available evidence demonstrates that *H. sanguineus* consistently has the upper hand in agonistic interactions within both regions. For example, studies in Long Island Sound demonstrate that juvenile *C. maenas* are outcompeted and reduce their use of intertidal rocks as low-tide refugia when *H. sanguineus* is abundant (Jensen et al., 2002). Further, *H. sanguineus* consumes settling *C. maenas* postlarvae within Long Island Sound, and this consumption has been credited for the decline of *C. maenas* in this region (Lohrer and Whitlatch, 2002). Competition is also important further north within the Gulf of Maine where there is a negative correlation in the presence and abundance of the two species at small, local scales (0.5 m) over which the crabs interact (Griffen et al., 2008). Additionally, interactions between these species in the Gulf of Maine cause *C. maenas*, a primarily carnivorous species, to switch the main component of its diet from mussels to algae. This results in a ~6% decrease in mussel consumption across sites for every one-crab increase in *H. sanguineus* density (e.g., from 5 to 6 crabs per  $m^{-2}$ ), even after accounting for differences across sites in mussel density and latitude. As a result, at sites where *H. sanguineus* was absent, mussels comprised up to 80% of *C. maenas*' diet, depending on mussel density, whereas when *H. sanguineus* density exceeded nine individuals  $m^{-2}$ , *C. maenas* consumed virtually no mussels, even when mussels were highly abundant (Griffen et al., 2008). The strong dependence of this diet shift on *H. sanguineus* density combined with greater *H. sanguineus* densities further south acts to strengthen this diet shift from north to south within the Gulf of Maine (Griffen et al., 2008).

Our purpose here is to revisit the potential mechanism(s) that lead to the elimination of *C. maenas* with the arrival of *H. sanguineus* at new sites and to provide evidence that the elimination of *C. maenas* with the arrival of *H. sanguineus* into the Gulf of Maine is occurring at a much slower time scale than occurred in Long Island Sound. Understanding the mechanistic basis of this interaction and the regional differences in its outcome has particular importance given that *C. maenas* is a prolific invader, having now invaded every continent except Antarctica. We focused our study on northern regions of the U.S. east coast where *C. maenas* and *H. sanguineus* are still both found abundantly and where examining frequent interactions between them is still possible.

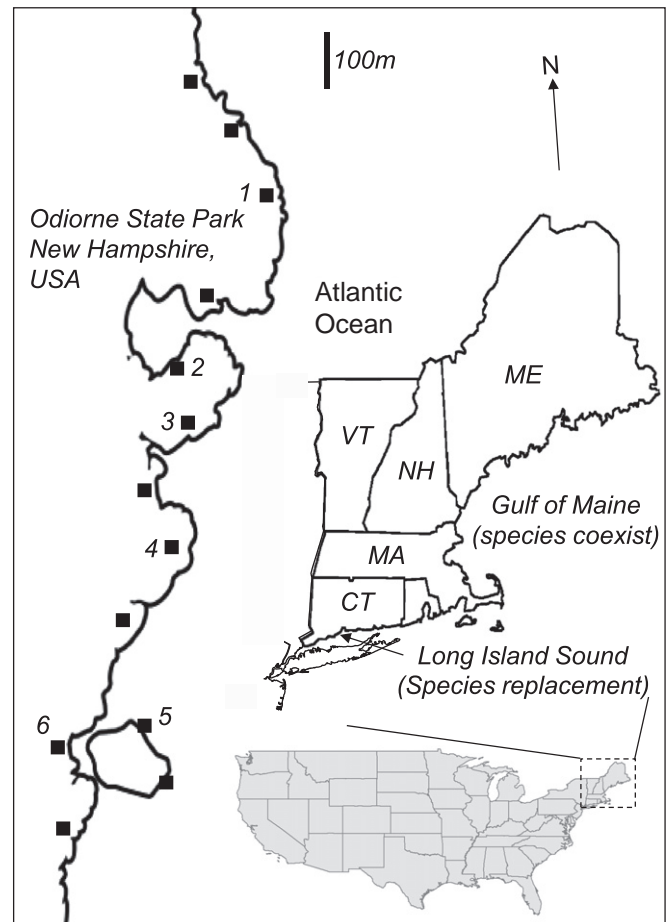
We take a two-pronged approach. First, we demonstrate that the rate of decline of *C. maenas* within the Gulf of Maine has been much slower than the documented decline in Long Island Sound. We did this by examining the densities of the two crabs at a single site in the southern Gulf of Maine where their interactions have been studied

extensively over the past decade, and for which data on species density over this time period therefore exists. Second, we explore the possibility that the documented diet shift by *C. maenas* in the presence of *H. sanguineus* from mussels to algae contributes to the demise of *C. maenas* through negative effects on energetics and reproduction. We also consider how this mechanism relates to the elimination of *C. maenas* in southern areas and how it could potentially lead to differential elimination of *C. maenas* in northern and southern regions.

## 2. Methods

### 2.1. Study site

*H. sanguineus* was first noted on the New Hampshire coast and at this site specifically in 1998 (Tyrrell and Harris, 1999), and has thus been present in this area for just over a decade. We conducted our study at Odiorne Point, NH, a semi-exposed site characterized by a series of coves (Fig. 1) that provides abundant intertidal boulder habitat that is heavily used by both *C. maenas* and *H. sanguineus* (Griffen et al., 2008). We chose to use Odiorne Point because the populations of these species have been studied extensively at this site since 1997, one year before the arrival of *H. sanguineus* (Tyrrell and Harris, 1999). This site therefore provides a good opportunity to examine how the *C. maenas* population has changed following the invasion of *H. sanguineus*. Additional work conducted at this site following the arrival of *H. sanguineus* has focused primarily on



**Fig. 1.** Map showing location of sampling areas at Odiorne Point on the New Hampshire coast that were sampled in 2005. Numbered sites (1–6) were resampled in 2009. Inset map shows broader area of interest indicating the Gulf of Maine where *Carcinus maenas* and *Hemigrapsus sanguineus* still coexist, and Long Island Sound where the invasion of *H. sanguineus* has resulted in species replacement.

interactions between these species, demonstrating negative effects of *H. sanguineus* on foraging success of *C. maenas* (Griffen, 2006; Griffen et al., 2008; Griffen and Williamson, 2008), and documenting and comparing the community impacts of the two species (Griffen and Byers, 2009).

## 2.2. Shifting abundances of *C. maenas* and *H. sanguineus*

We used data on the abundance and size frequency distribution of both crab species sampled during low tide at Odiorne Point to assess the evidence that *C. maenas* at this site is being replaced by *H. sanguineus*. All samples were collected using identical methods from approximately 0.5 m above mean lower low water using haphazardly placed 0.5 m<sup>2</sup> quadrats. Each quadrat was sampled by removing all boulders and cobble from within the quadrat, and collecting, determining the gender, and measuring the carapace width of all crabs to the nearest 0.1 mm. Fifteen replicate quadrats were sampled from each year or from each cove (explained below), with the exception of the sample in 2003 when only six quadrats were sampled.

We assessed whether *C. maenas* density is declining and being replaced by *H. sanguineus* at Odiorne Point using two comparisons. We first compared changes in the abundance of the two crab species through time using a series of samples collected in June of each year from a single stretch of beach within Odiorne Point during the years 2003, 2004, 2005, 2009, and 2010 (site #4 in Fig. 1). We initially compared changes in density across years using ANCOVA, with the density of crabs in individual quadrats as the response variable, species treated as the main effect and year as the covariate. However, this initial analysis resulted in a significant interaction term between the main effect and covariate, indicating that the two species differed in their trends across years. We therefore performed separate linear regressions for each species in order to determine how each varied across years. We also qualitatively compared the density of *C. maenas* during this time interval (2003–2010, i.e., post-*H. sanguineus* invasion) to a single estimate of mean *C. maenas* density reported at Odiorne Point in 1997 (Tyrrell and Harris, 1999), the year before the arrival of *H. sanguineus* at this site.

The density of both *C. maenas* and *H. sanguineus* across 13 separate coves at Odiorne Point in 2005 was previously reported (Griffen et al., 2008). The second comparison examined how the density of the two species varied across six of these coves (randomly chosen) that we re-sampled in 2009 (Fig. 1). We compared the density across these coves in 2005 and 2009 using a mixed-model ANOVA with the density in individual quadrats as the response variable, species and year as fixed factors and cove as a random factor. The data across sites were log-transformed prior to analysis to meet the assumption of homogeneity of variances. These and all other analyses were conducted using R v.2.7.1. The final sampling period for both of these comparisons (2010 and 2009, respectively) was 13 and 12 years after the arrival of *H. sanguineus* at this site (Tyrrell and Harris, 1999), which is 3–4 times longer than the time required for the elimination of *C. maenas* from sites in Long Island Sound following the arrival of *H. sanguineus* (Kraemer et al., 2007; Lohrer and Whitlatch, 2002).

In addition, we used these same data to compare differences between 2005 and 2009 in the size-frequency distribution of the two species. To do this we grouped individuals by carapace width in 5 mm increments: ≤5, 6–10, 11–15, etc. We then visually compared graphs of size-frequency distributions during 2005 and 2009 for each species separately. The rationale for this comparison is that if the *C. maenas* population is declining at this site due to predation on its recruits or lack of recruitment, then it should be characterized by a size-frequency distribution that is shifted toward larger individuals. Conversely, if the *H. sanguineus* population is increasing, then it should be characterized by a size-frequency distribution that is shifted toward smaller individuals, consistent with abundant recruitment.

## 2.3. Effects of *C. maenas* diet shift on energetics and reproductive potential

In the presence of *H. sanguineus*, *C. maenas* switches from consuming primarily animal tissue to consuming primarily algae, and this effect becomes stronger as *H. sanguineus* density increases (Griffen et al., 2008). We examined the implications of this diet shift for *C. maenas* reproduction using a two-pronged approach. First, we experimentally manipulated diet and reproductive state to determine how diet influences reproductive potential and energy storage during both reproductive and nonreproductive periods. Second, we used stable isotopes and physiological measurements to determine the relationship between diet, reproduction, and energy storage in crabs under natural conditions.

Two aspects of crab biology are pertinent to our methods. First, reproduction in decapod crustaceans is under the control of gonad-inhibiting hormone that is produced from a gland located in the eyestalk (Adiyodi and Adiyodi, 1970). In many crabs, including *C. maenas*, removal of the eyestalk (eyestalk ablation) eliminates this hormone and therefore stimulates vitellogenesis and the extrusion of eggs within 2–3 weeks (Bliss, 1966; Demeusy, 1962). Second, energy and nutrients required for molting and reproduction in crustaceans are stored in the hepatopancreas (Kennish, 1997). However, previous work with *C. maenas* did not find any seasonal change in the size or composition of the hepatopancreas, as may be expected if *C. maenas* is withdrawing energy or nutrients from the hepatopancreas for gonad development or egg production (Heath and Barnes, 1970). It is therefore unclear whether *C. maenas* obtains energy/nutrients for reproduction from stores held in the hepatopancreas, or from food intake during vitellogenesis.

### 2.3.1. Experimental manipulation of diet and reproductive state

We experimentally imposed both diet differences and eyestalk ablation to examine how diet influenced reproductive capacity and energy storage both during reproduction and during non-reproductive periods. We collected 24 female *C. maenas* (CW = 40.7 ± 4.2 mm, mean ± SD) intertidally from Odiorne Point in June 2009. By June in the southern Gulf of Maine, most reproductive female *C. maenas* are either ovigerous or have already released their clutch (Berrill, 1982). To ensure that we did not include the occasional crab that was still in the process of vitellogenesis, we only used crabs that had a green carapace, indicating that they had recently molted (Styrishave et al., 2004) and were therefore not engaged in vitellogenesis (Reid et al., 1994). We performed unilateral eyestalk ablation on half of the crabs. We then randomly assigned each crab to one of three diet treatments: mussels (*Mytilus edulis*), algae (*Ulva lactuca*), or a mixed diet of both mussels and algae. Fresh food was provided in excess daily and any uneaten food from the previous day was removed. To ensure that crabs in the mixed diet treatment consumed both food types, only a single food type was offered each day, and mussels and algae were alternated on a daily basis. Crabs were housed individually in 500 ml polypropylene containers with 0.5 cm mesh sides and all containers were placed in a steel mesh cage that was suspended at 1 m depth from a floating dock on the New Hampshire coast. Crabs were maintained under these conditions for 21 days, after which we dissected each and determined the proportion of the body weight that was allocated to the gonads (gonadosomatic index, GSI) and the proportion of body weight that was allocated to the hepatopancreas (hepatosomatic index, HSI) (Kennish, 1997). We analyzed the results using separate linear models for GSI and HSI, with ablation (yes/no) and food (mussels, algae, mixed) as fixed factors in each analysis. Crab size is integrated within GSI and HSI. However, to ensure that size did not have an effect on experimental results (e.g., by inadvertently including larger individuals in the ablation treatments or in the mussel food treatment), we also included carapace width as a covariate in each of these analyses.

### 2.3.2. Diet, reproduction, and energy storage in crabs under natural conditions

We explored the correlation between diet and amount of reproduction in *C. maenas* at Odiorne Point using stable isotopes. We dissected 325 reproductive-size ( $40.1 \pm 5.7$  mm CW) female *C. maenas* collected from Odiorne Point between April 27 and 29, 2009. This is just before the peak in timing of ovigerous females in the southern Gulf of Maine, which occurs in May and June (Audet et al., 2008). Prior to dissection, crabs were stored at  $-80$  °C.

We performed stable isotope analysis ( $^{15}\text{N}$ ) on a subset of 202 of the sampled crabs using dried muscle tissue from walking legs. Several potential plant and animal food sources are abundant at Odiorne Point (Tyrrell and Harris, 1999). We therefore also analyzed triplicate samples of several of the most abundant plant (red algae: *Chondrus crispus*, *Polysiphonia lanosa*; brown algae: *Ascophyllum nodosum*, *Fucus vesiculosus*; and green algae: *Ulva lactuca*) and animal prey sources (mussels: *Mytilus edulis*; barnacles: *Semibalanus balanoides*; snails: *Littorina littorea*, *Littorina obtusata*, *Nucella lapillus*; urchins: *Strongylocentrotus droebachiensis*; and amphipods: *Gammarus* sp) at Odiorne Point. Samples were processed using an Isoprime mass spectrometer connected via continuous flow to a EuroVector Elemental Analyzer. Three replicate samples of an internal standard (USGS40) were run approximately every 40 samples to calibrate the system and to compensate for potential drift with time. The precision for these standards was better than  $\pm 0.07\%$ .

*C. maenas* is omnivorous, consuming a mix of plant and animal tissues. This can make diet analysis with stable isotopes particularly challenging. For this reason, our goal here was not to specify the precise diet of individual crabs, but rather, to determine the relative degree of carnivory vs. herbivory.  $\delta^{15}\text{N}$  values for animal prey at Odiorne were generally higher than values for plants (see Results section). We therefore used  $\delta^{15}\text{N}$  values of individual crabs as a proxy for the relative consumption of animal vs. plant tissue (i.e., a continuous measure of relative trophic level, Post, 2002). In addition, the level of gonad development is influenced by the time since the last molt (Styrishave et al., 2004), and relative amount of time since last molt in *C. maenas* can be determined by carapace color (Reid et al., 1997). Recently molted crabs are green, and with length of time since their last molt they first turn green-brown, then brown, followed by brown-red, and ultimately to red. We determined the color of each crab using the outer side of the first maxillae. The color of each crab was determined by the same observer in order to standardize observations to the extent possible. We therefore examined the influence of relative trophic level ( $\delta^{15}\text{N}$ , continuous variable) and carapace color (fixed factor with five levels) on GSI using ANCOVA. We also included crab size (CW) as a covariate in order to control for potential differences in GSI with crab size (e.g., due to possible increases in carnivory with crab size). A significant positive relationship between  $\delta^{15}\text{N}$  and GSI after accounting for carapace color and crab size would indicate a positive correlation between animal consumption and reproductive output and therefore would support the hypothesis that the shift from animal to plant consumption previously reported (Griffen et al., 2008) has a negative impact on reproductive output.

In addition to the above analyses, we examined the correlation between the GSI and HSI for the sampled crabs to provide insight into whether energy/nutrients required for egg production is derived from stores in the hepatopancreas or is derived from consumption during vitellogenesis. We therefore restricted this analysis to those crabs that were undergoing vitellogenesis at the time of collection ( $n = 123$ ). Molting and reproduction are intricately related, but mutually exclusive events in crustaceans (Raviv et al., 2008). Vitellogenesis may have begun at different lengths of time following the last molt, and energy stores (HSI) generally accumulate between molts. We therefore controlled for time since molt in the analysis by again including carapace color as a fixed factor with five levels (ANCOVA). A

significant negative relationship between GSI and HSI after accounting for time since molting (carapace color) in this analysis would suggest that *C. maenas* withdraws energy stored in the hepatopancreas for use in reproduction. Results of this analysis were used to help interpret findings of the experiment described above that manipulated diet and reproductive state.

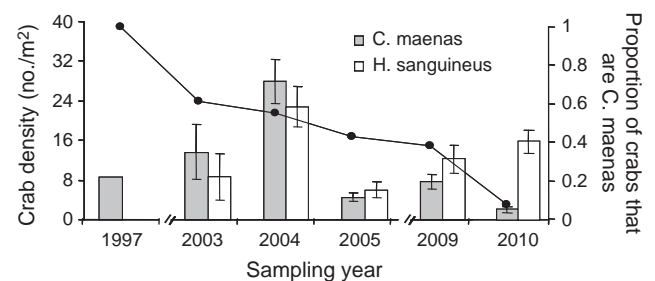
## 3. Results

### 3.1. Shifting abundances of *C. maenas* and *H. sanguineus*

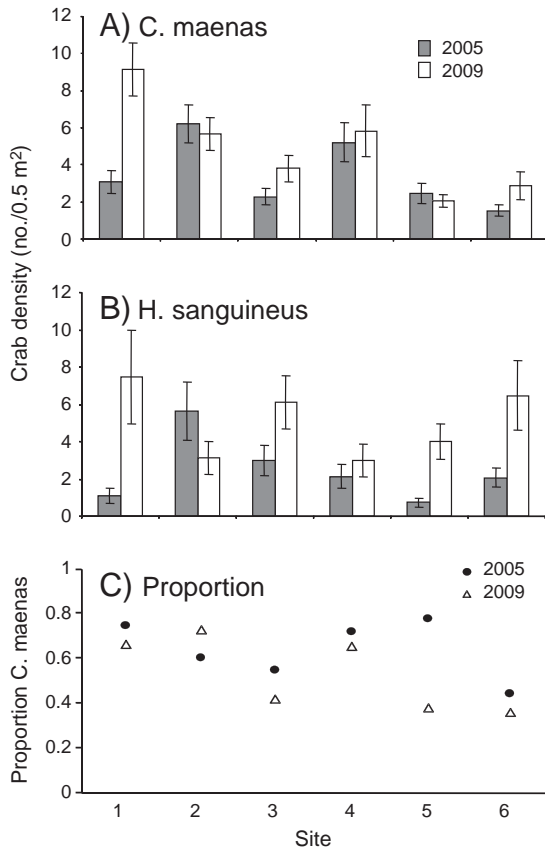
Variation in the density of *H. sanguineus* across years at Odiorne Point is high. However, in contrast to trends reported at sites within Long Island Sound, there does not appear to be a trend over time toward increasing densities (linear regression,  $t = 1.67$ ,  $df = 64$ ,  $P = 0.10$ , Fig. 2). In contrast, there was a decrease in the density of *C. maenas* during the time interval from 2003 to 2010 (linear regression,  $t = -4.29$ ,  $df = 64$ ,  $P < 0.0001$ , Fig. 2). As a result, since the arrival of *H. sanguineus*, *C. maenas* has comprised a continually decreasing proportion of the crabs at this site (Fig. 2). For comparison, we also show the density of *C. maenas* at this tidal height at Odiorne Point in 1997 before the arrival of *H. sanguineus* (left bar in Fig. 2, taken from Tyrrell and Harris, 1999). Although Tyrrell and Harris's study took place along the same stretch of intertidal shore as our work, the precise location of their sampling is unknown and therefore care should be taken when making direct comparison with the data presented. However, given the generally similar sampling locations, this data provides no evidence that *C. maenas* experienced rapid declines in densities within the year or two following the arrival of *H. sanguineus*.

While *C. maenas* appears to be declining when viewed across several years at a single site as described in the preceding paragraph, we see a different trend when we compare across several sites for 2005 and 2009. Specifically, we found higher overall densities of both crab species in 2009 (*C. maenas*:  $4.9 \pm 4.4$ , mean  $\pm$  SD; *H. sanguineus*:  $5.0 \pm 6.0$ ) than in 2005 (*C. maenas*:  $3.5 \pm 3.1$ ; *H. sanguineus*:  $2.4 \pm 3.5$ ) (mixed effects model, main effect of year,  $t = 3.47$ ,  $d.f. = 336$ ,  $P = 0.0006$ , Fig. 3). Small density differences between the two species were not, however, significant in either year (main effect of species,  $t = 0.49$ ,  $d.f. = 336$ ,  $P = 0.63$ ; year  $\times$  species interaction,  $t = 0.48$ ,  $d.f. = 336$ ,  $P = 0.63$ , Fig. 3). As was reported previously for the 2005 data (Griffen et al., 2008), there were differences in the densities of the two species across the six coves and the density in each cove differed across the two years (main effect of site and site  $\times$  year interactions for each site,  $P < 0.05$ , Fig. 3).

The size distribution of *C. maenas* was strongly skewed toward small juveniles in both years (Fig. 4A and B). For *H. sanguineus*, the size distribution differed across the two years, with adults dominating the population in 2005 (Fig. 4C), and many more juveniles in 2009



**Fig. 2.** Density of *Carcinus maenas* and *Hemigrapsus sanguineus* at a single site at Odiorne Point, NH (site 4 in Fig. 1) from 2003 to 2010 (bars, left axis, values are mean  $\pm$  SE). Proportion of crabs at each site for each year that were *C. maenas* (dots and line, right axis). Proportion of crabs is made up of *C. maenas* and *H. sanguineus* only, as these were overwhelmingly the numerically dominant crab species at this site.



**Fig. 3.** Density of *Carcinus maenas* (part A) and *Hemigrapsus sanguineus* (part B) and proportion of crabs that were *C. maenas* (part C) at six sites (coves) within Odiorne Point, NH in July of 2005 and June of 2009. Values are mean  $\pm$  SE ( $n = 15$ ). Site numbers on x-axis correspond to numbered sites in Fig. 1.

(Fig. 4D), suggesting that recruitment of *H. sanguineus* is episodic, with good and bad years at this site.

### 3.2. Effects of *C. maenas* diet shift on energetics and reproductive potential

#### 3.2.1. Experimental manipulation of diet and reproductive state

Our experiment demonstrated that shifting from a mussel to an algal diet reduces reproduction and energy stores in the hepatopan-

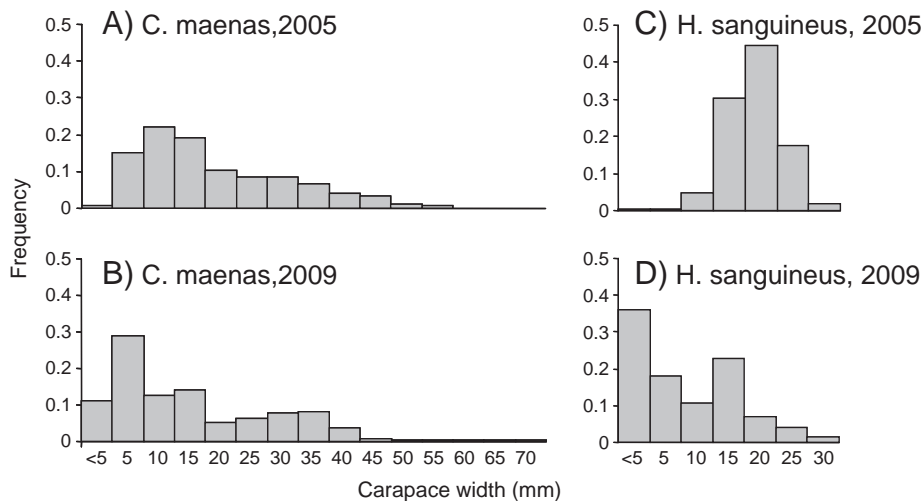
creas. As expected, we found that eyestalk ablation stimulated gonad development and resulted in larger GSI for ablated than for unablated crabs (ANOVA,  $F_{1,12} = 17.68$ ,  $P = 0.001$ , Fig. 5A). Mussel consumption also had a positive impact on gonad development relative to algal or mixed diets ( $F_{2,12} = 4.99$ ,  $P = 0.03$ , Fig. 5A). Conversely, eyestalk ablation had no impact on the hepatopancreas and there was therefore no difference in HSI of ablated and unablated individuals (ANOVA,  $F_{1,18} = 0.21$ ,  $P = 0.66$ , Fig. 5B), while mussel consumption did lead to increased allocation of mass to the hepatopancreas relative to crabs that ate an algal or a mixed diet (ANOVA,  $F_{2,18} = 7.21$ ,  $P = 0.009$ , Fig. 5B). GSI and HSI were not influenced in our experiment by crab size ( $P > 0.15$  in both analyses). None of the interaction effects (between CW, diet, and ablation) were significant for either analysis (all  $P > 0.2$ ).

#### 3.2.2. Diet, reproduction, and energy storage in crabs under natural conditions

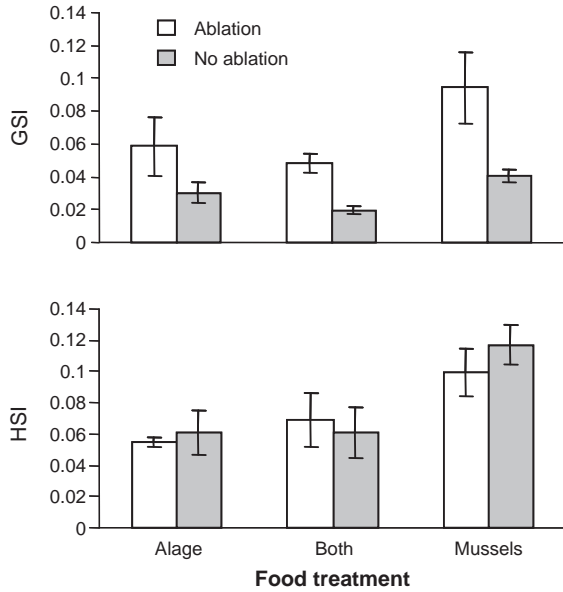
Potential animal prey of *C. maenas* were enriched in  $\delta^{15}\text{N}$  values ( $8.409 \pm 1.483\%$ , mean  $\pm$  SD) relative to plants ( $6.142 \pm 1.372\%$ ) at Odiorne Point ( $t$ -test on mean values, d.f. = 13,  $t = 3.659$ ,  $P = 0.001$ ).  $\delta^{15}\text{N}$  values of individual *C. maenas* indicated a broad range of trophic levels for crabs at this site ( $\delta^{15}\text{N}$  ranged from 9.932 to 13.516), suggesting varying individual dietary strategies from primarily herbivorous to primarily carnivorous (assuming fractionation of approximately 3.4‰ with each trophic level, Fry, 2006). Further, trophic level was positively correlated with reproductive output (GSI) ( $\delta^{15}\text{N}$  covariate in ANCOVA,  $F_{1,186} = 9.72$ ,  $P = 0.002$ , Fig. 6). This was the case even after accounting for the strong positive effect of time since last molt on GSI (fixed effect of carapace color,  $F_{4,186} = 40.30$ ,  $P < 0.0001$ , Fig. 6) and crab size (CW covariate,  $F_{1,186} = 53.03$ ,  $P < 0.0001$ ). Consistent with energy requirements for reproduction coming from the hepatopancreas, we also found a negative correlation between relative gonad mass (GSI) and relative hepatopancreas mass (HSI) in vitellogenic crabs (ANCOVA covariate,  $F_{1,117} = 4.30$ ,  $P = 0.04$ , Fig. 7), even after accounting for the positive effect of time since last molt on HSI (ANCOVA fixed effect,  $F_{4,117} = 7.84$ ,  $P = 0.0001$ ).

## 4. Discussion

Contrary to the pattern observed in Long Island Sound where the arrival of *H. sanguineus* resulted in the rapid decline of *C. maenas* within 2–3 years (Kraemer et al., 2007; Lohrer and Whitlatch, 2002), our results demonstrate that the arrival of *H. sanguineus* in northern sites, within the Gulf of Maine, has coincided with a much slower decline of *C. maenas* (Figs. 2 and 3). This regional difference in the

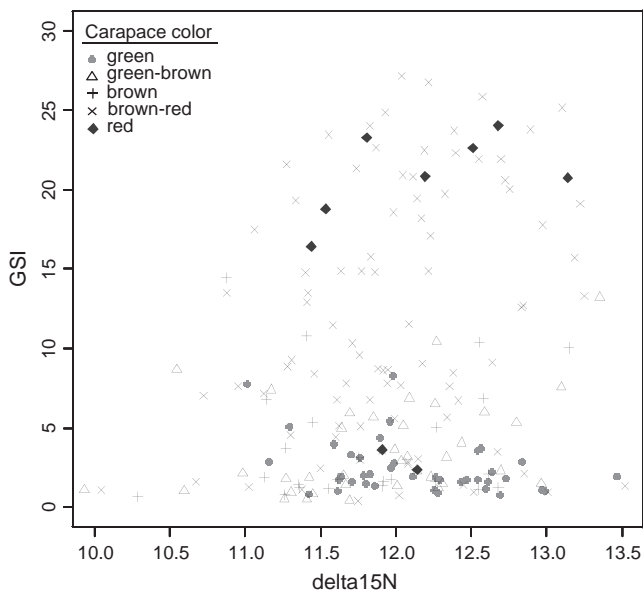


**Fig. 4.** Size frequency distribution of *Carcinus maenas* and *Hemigrapsus sanguineus* at Odiorne Point, NH in 2005 and 2009. Data are from all six coves at Odiorne Point, NH combined.

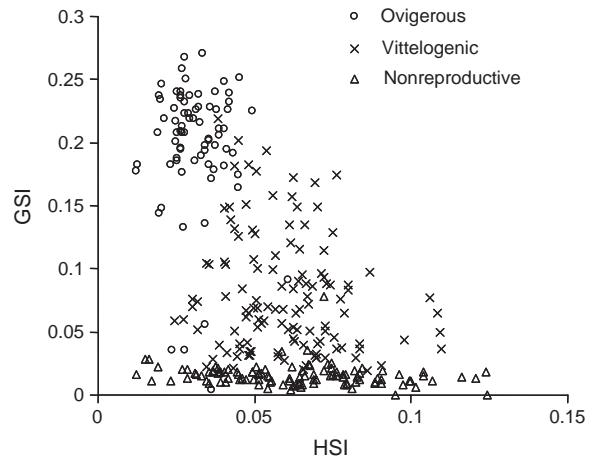


**Fig. 5.** Effects of experimental diet on reproductive effort (gonadosomatic index, GSI) and energy stores (hepatosomatic index, HSI) for *Carcinus maenas* both with and without eyestalk ablation.

effects of *H. sanguineus* could be due to a difference in the strength of predation on *C. maenas* larvae, possibly due to habitat differences between the two regions. Our data does not rule out this possibility; however, our findings suggest another contributing factor to *C. maenas*' decline. Specifically, we find that small *C. maenas* have less energy stores and reduced gonad development coinciding with a dietary shift toward algae. In another study, this diet shift was experimentally attributed to the presence of *H. sanguineus* (Griffen et al., 2008). Taken together, these findings may be contributing to different rates of *C. maenas* decline in northern and southern areas. These two mechanisms (predation and reduced reproduction) are not mutually exclusive and we discuss each below.



**Fig. 6.** Correlation between  $\delta^{15}\text{N}$  and GSI and between  $\delta^{15}\text{N}$  and carapace color. Recently molted crabs are green, and with length of time since their last molt they first turn green-brown, then brown, followed by brown-red, and ultimately to red.



**Fig. 7.** Tradeoff between energy invested in reproduction (GSI) and energy storage (HSI) for *Carcinus maenas* sampled from Odiorne Point demonstrating that energy used for egg production is likely taken from the hepatopancreas.

#### 4.1. Regional difference in *H. sanguineus* predation

There could potentially be a regional difference in the importance of predation by *H. sanguineus* on *C. maenas*. At sites within Long Island Sound, predation by *H. sanguineus* reduced recruitment of *C. maenas* by 50–75% (Fig. 5 of Lohrer and Whitlatch, 2002). Potential differences in predation by *H. sanguineus* between these two regions could be influenced by three interrelated factors: the amount of intertidal refuge, the temperature and related influences on consumption rates, and the density of *H. sanguineus*. As stated previously, rocky habitat that provides important intertidal refuge for *H. sanguineus* at low tide is the dominant habitat in the Gulf of Maine, whereas its relative prominence is much lower in Long Island Sound. As a result, *H. sanguineus* populations in the Gulf of Maine can disperse more than populations in Long Island Sound that are forced to aggregate into smaller patches of cobble and boulder habitat. This aggregation may be responsible for the high densities of *H. sanguineus* that have been reported in Long Island Sound (90, 150, and even 300 crabs  $\text{m}^{-2}$  reported by Brousseau et al., 2003; Kraemer et al., 2007; Lohrer and Whitlatch, 2002). Density differences in the two regions may also result from different levels of recruitment of *H. sanguineus* in the two regions. Comparison of *H. sanguineus* size-frequency distributions in 2005 and 2009 (Fig. 4C and D) suggests that recruitment at Odiorne Point is much higher in some years (2009) than others (2005) and while no comparable data is available for Long Island Sound, it is possible that *H. sanguineus* recruitment in Long Island Sound is higher and/or more consistent. Regardless of the underlying reason for density differences, higher population densities in southern areas likely lead to higher intensity of interactions, such as predation. Recruiting *C. maenas* preferentially settle into complex habitat, such as cobble and rocky intertidal sites (Hedvall et al., 1998). Thus, high densities of *H. sanguineus* in these habitats within Long Island Sound present a gauntlet of potential predators into which *C. maenas* must settle. The situation in the Gulf of Maine is quite the opposite, suitable settlement habitat is more abundant and *H. sanguineus* densities are much lower, potentially allowing *C. maenas* to settle under much lower risk of predation.

#### 4.2. Reduced reproduction resulting from the diet switch from mussel to algal consumption

While *H. sanguineus* clearly consumes *C. maenas* (Lohrer and Whitlatch, 2002), diet analysis from crabs collected around New

Jersey suggests that crustaceans are not a major component of *H. sanguineus*' diet (McDermott, 1998b). Evidence for the importance of predation in eliminating *C. maenas* from sites in Long Island Sound comes from the absence of *C. maenas* recruits in experimental plots containing *H. sanguineus* (Lohrer and Whitlatch, 2002). The absence of *C. maenas* can conceivably be explained by other factors, such as avoidance of *H. sanguineus*-containing cages by settling *C. maenas* (Hedvall et al., 1998) or post-settlement migration out of cages containing *H. sanguineus* (Moksnes, 2002). Thus predation by *H. sanguineus* may not be the only factor responsible for the elimination of *C. maenas*. An additional contributing mechanism may be reductions in reproductive success stemming from an altered diet in the presence of *H. sanguineus*. There is a latitudinal trend in *C. maenas*' diet, with mussel consumption decreasing from north to south as *H. sanguineus* becomes more dense, even in sites where mussels are readily available (Griffen et al., 2008). This diet shift appears to be the direct result of interactions between the two species (Griffen et al., 2008). Results here demonstrate that this diet shift can reduce reproductive potential.

We found that crabs that were experimentally restricted to either a complete algal diet or a diet that was half algae for just three weeks had 40–50% lower reproductive potential and accumulated 40–50% less mass in their hepatopancreas than crabs that were fed a diet of mussels only (Fig. 5). Much larger impacts may potentially be expected if diet shifts are maintained throughout the foraging season. This experimental trend was supported by evidence from field data where crabs that consumed more plants (had lower  $\delta^{15}\text{N}$ ) had lower reproductive potential (GSI). The negative correlation between the GSI and HSI in our field data suggests that energy for reproduction in *C. maenas* is drawn from stores in the hepatopancreas and therefore also supports the conclusions that reduced storage in the hepatopancreas resulting from this diet shift may subsequently lead to lower future reproductive output (Figs. 5–7).

The mechanism underlying the reduced reproductive potential following the shift from a mussel to an algal diet may be nitrogen limitation. Crabs that eat a substantial amount of algae are generally nitrogen limited (Wolcott and O'Connor, 1992). In fact, crabs that are primarily herbivorous often cannot reproduce due to nitrogen limitation without first supplementing their herbivorous diet with a minimum level of N-rich animal consumption (Kennish, 1996). The full reproductive implications of this diet shift should therefore depend on the species of algae that are consumed in lieu of mussel consumption. We chose to use the green alga *Ulva lactuca* in our experiment because this is the preferred plant food in laboratory food choice trials (Griffen, 2011). However, because it is relatively scarce, this species comprises only a minor portion of *C. maenas*' natural diet in rocky intertidal areas. Instead, the more abundant but relatively less nutritious red alga *Chondrus crispus* comprised  $\sim 81\% \pm 33\%$  of the plant material found in the gut of adult female *C. maenas* across 15 sites in the southern Gulf of Maine (Griffen, unpubl. data,  $n = 118$ ). Mussels are approximately an order of magnitude richer in nitrogen than *C. crispus*. Thus, a diet shift from mussels (7.7%–9.6% nitrogen, Smaal and Vonck, 1997) to *C. crispus* (0.4%–1.4% nitrogen, Chopin and Floch, 1992) may have an even stronger effect than our experimental diet using *U. lactuca* (4.1%–5.5% nitrogen, Cohen and Neori, 1991).

If reproductive limitation is a factor contributing to the decline of *C. maenas*, its effects are likely very different within the Gulf of Maine and within Long Island Sound due to differences in exogenous larval supply. Cape Cod, which separates these two regions, represents a biogeographic boundary (Bertness et al., 1999) where different current patterns north and south of this boundary result in the delivery of different sets of larval recruits, with different resulting faunal assemblages. Over the last several decades *C. maenas* has expanded its range northward and has now established populations as far north as the Gulf of St. Lawrence, Canada (Audet et al., 2003). Within the Gulf of Maine, the Eastern and Western Maine Coastal

Currents transport *C. maenas* larvae produced in areas far north of *H. sanguineus*' range into the southern Gulf of Maine. *C. maenas*' shift from mussel to algal consumption becomes more pronounced toward southern areas in the Gulf of Maine, where *H. sanguineus* densities are the highest (Griffen et al., 2008). However, even if *C. maenas* in the southern Gulf of Maine did not reproduce at all, *C. maenas* would likely still recruit to this area as a result of production in upstream areas of the northern Gulf of Maine. In contrast, populations of *C. maenas* within Long Island Sound receive no such subsidy, largely because populations of *C. maenas* are limited in the southern portions of its range (the southern upstream region, via the Gulf Stream Current) by aggressive interactions with the native blue crab *Callinectes sapidus* (deRivera et al., 2005). Thus, the very rapid declines in *C. maenas* density with the arrival of *H. sanguineus* into Long Island Sound could reflect relatively closed populations that experience reproductive failure from the observed diet shift. The much slower *C. maenas* declines observed in the Gulf of Maine on the other hand may reflect reductions by the same mechanism, but with open populations receiving continued supplemental recruitment from more northern regions that are free of interactions with *H. sanguineus*. Given this ongoing subsidy, *C. maenas* may never entirely disappear from rocky intertidal habitats within the Gulf of Maine.

Finally, large *C. maenas* commonly move into subtidal habitats where the extent to which they interact with *H. sanguineus* is unknown (Hunter and Naylor, 1993). Additionally, the relative contribution of intertidal and subtidal females to overall population reproduction is unclear. However, if subtidal *C. maenas* interact less with *H. sanguineus*, then subtidal females may provide another source of recruits that enable the persistence of *C. maenas*, even if reproduction by smaller intertidal females were entirely eliminated. Subtidal refuge may be less important in Long Island Sound where interactions with blue crabs limit *C. maenas* (deRivera et al., 2005). Regardless of this potential role of large subtidal individuals, *C. maenas* generally first passes through an extended period of intertidal residence where it directly interacts with *H. sanguineus*. Our study has demonstrated an additional mechanism by which this interaction may limit the success of *C. maenas*.

#### 4.3. Conclusions

Examining regional variation in species interactions can provide novel insights into ecological and evolutionary questions (Travis, 1996). We have demonstrated that the outcomes of interactions between two invasive crabs in the Gulf of Maine are somewhat different from outcomes reported for interactions between the same two species in Long Island Sound. These differences are likely influenced by marked environmental differences that occur north and south of Cape Cod. Such changes in species interactions may be common at biogeographic boundaries, near the edge of species' ranges, or as a result of anthropogenic habitat changes. In addition to spatial differences, species interactions may differ with temporally variable environmental drivers (e.g., seasonal, ENSO). As our study demonstrates, understanding how species interactions vary both spatially and temporally is fundamental to accurately assessing the interactions with widespread invasive species.

#### Acknowledgments

Thanks to M. Griffen for assistance in creating Fig. 1. This work was funded by the University of South Carolina. [RH]

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