Journal of Experimental Marine Biology and Ecology 466 (2015) 70–75

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Rhizocephalan infection modifies host food consumption by reducing host activity levels

Benjamin A. Belgrad [a,*], Blaine D. Griffen [a,b,1,2]

[a] School of Earth, Ocean, and Environment, Department of Marine Science, University of South Carolina, 701 Sumter Street, EWS 617, Columbia, SC 29208, United States
[b] Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, United States

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 12 February 2015
Accepted 13 February 2015
Available online 25 February 2015

Keywords:
Behavioral modification
Community dynamics
Eurypanopeus depressus
Microhabitat
Parasitism

ABSTRACT

Although castration by rhizocephalans on crab species is well documented, the accompanying effects of infection on behavior and metabolism have remained relatively unstudied. In this investigation, we examined flat back mud crab (*Eurypanopeus depressus*) physiology and behavior in an attempt to elucidate why infected crabs exhibit a previously documented reduced functional response. Crab respiration and digestion rates were analyzed to determine if infection altered metabolic rate. Laboratory behavioral experiments and a field survey were conducted to determine how infection alters crab feeding behavior and activity levels. Although we found no statistical difference between infected and uninfected crab metabolic or digestive rates, we discovered that, both in the lab and in the field, infected crabs exhibited substantially altered behavior. In the laboratory infected crabs reacted nearly 3 times slower to the presence of prey and spent over 22% more of their time hiding, whereas uninfected crabs were significantly more active. During field sampling, infected crabs were significantly more likely to be found hiding within empty oyster shells while uninfected crabs spent more time in the exposed positions of the habitat. We conclude that rhizocephalans can reduce the host functional response by altering host behavior. Here, these induced changes can impact community structure by altering trophic interactions so that infected crabs spend less time foraging and more time hiding, potentially reducing their predation risk.

1. Introduction

Parasites are well known for manipulating host behavior (Belgrad and Smith, 2014; Hindsbo, 1972; Moore, 2002; Poulin, 1995; Poulin, 2010). Classical examples include acanthocephalans altering amphipod phototactic behavior to increase their predation risk by mallard ducks (Bethel and Holmes, 1973, 1977) and the trematode *Dicrocoelium dendriticum* inducing infected ants to climb blades of grass consumed by sheep (Carney, 1969). Other common host behaviors modified by parasites include foraging behavior (Koella et al., 1998), sexual behavior (Dunlap and Schall, 1995; Vance, 1996), activity levels (Moore, 2002; Webster, 1994), and habitat selection (Belgrad and Smith, 2014; MacNeil et al., 2003).

Such changes to behavior are significant because they directly impact host fitness (Fitze et al., 2004; Marzal et al., 2005; Yanoviak et al., 2008) and indirectly influence community structure (Minchella and Scott, 1991; Mouritsen and Poulin, 2005). For example, killifish infected by trematodes more frequently exhibit conspicuous behaviors such as jerking motions, and are subsequently more susceptible to predation from birds (Lafferty and Morris, 1996). Correspondingly, isopods infected with acanthocephalans are more active and frequent light-colored exposed substrates more than uninfected isopods, and subsequently infected isopods were preferentially consumed by starlings in the laboratory (Moore, 1983). Behavioral changes induced by parasites can impact community structure by altering trophic interactions either through manipulating the host functional response (Toscano et al., 2014; Wood et al., 2007) or the hosts' predation risk (Minchella and Scott, 1991).

One type of parasite recognized for altering decapod host sexual behavior and population dynamics is the rhizocephalan barnacle (Mouritsen and Poulin, 2002; Reinhard, 1956; Sloan, 1984). Rhizocephalans are extremely well-adapted cirripeds, which solely require crustaceans, principally crabs, as hosts (Hoeg, 1995). Parasitic infection begins when a female cyprid larva settles on either a male or female crab and grows a system of branching roots along the intestines of the crab. This initial stage, called the interna, resides entirely within the host (Alvarez et al., 1995; O'Brien and Van Wyk, 1985; Walker et al., 1992). Eventually an externa is produced as the rhizocephalan matures, and a portion of the parasite erupts under the abdomen of the crab. The externa comprises the reproductive body of the parasite and resides in the same location that normally would be occupied by the egg mass of an uninfected brooding female decapod. The externa

* Corresponding author. Tel.: +1 330 398 3900.
E-mail addresses: babelgra@eckerd.edu (B.A. Belgrad), bgriffen@biol.sc.edu (B.D. Griffen).
[1] Tel.: +1 330 398 3900.
[2] Tel.: +1 803 603 9532.

http://dx.doi.org/10.1016/j.jembe.2015.02.011
0022-0981/

will remain a small bud until a male cyprid larva fertilizes the externa (O'Brien and Van Wyk, 1985). In addition to castration for both male and female crabs, rhizocephalans produce a variety of effects in their hosts, ranging from reduced growth rates (O'Brien and Van Wyk, 1985) to altered aggression (Innocenti et al., 2003) and burrowing behavior (Innocenti et al., 1998; Mouritsen and Jensen, 2006).

The invasive rhizocephalan barnacle, *Loxothylacus panopaei*, infects and castrates the flat-backed mud crab, *Eurypanopeus depressus*, and other xanthid crabs along the Atlantic coast of North America (Alvarez et al., 1995). Originally a native of the Gulf of Mexico, *L. panopaei* began to invade the eastern coast of the United States in 1964, presumably through the importation of oysters from the Gulf of Mexico, which likely bore infected crabs (Van Engel et al., 1966). The rhizocephalan has a prevalence of between 8% and 29% depending on the month, within North Inlet estuary in South Carolina (O'Shaughnessy et al., 2014; Toscano et al., 2014), but can reach as high as 90% in its introduced range (Hines et al., 1997; Kruse and Hare, 2007). Recently, Toscano et al. (2014) found that mature *L. panopaei* reduces the functional response of *E. depressus*, limiting the amount of mussels the crab consumes. In contrast, some studies on animal feeding behavior found that parasitized hosts often increased their consumption (Barber et al., 2000; Koella et al., 1998). Similarly, infected crabs may also be expected to consume more mussels to meet the increased energetic demands associated with supporting such an intrusive parasite. We therefore sought to determine what underlying factors caused infected *E. depressus* to exhibit a reduced functional response.

Several mechanisms could potentially explain the decreased consumption by infected crabs. First we hypothesized that decreased mussel consumption by crabs could be the product of a reduced metabolic rate (i.e., decreased energy needs), which could occur if rhizocephalans reduce host energy expenditures on growth or movement. Second, rhizocephalans may decrease the digestive rate (i.e., decreased food processing capability) of crabs since the parasite interna infests the crab body and may be damaging or reducing the efficiency of the digestive tract. Third, rhizocephalans may indirectly lower the consumption rates of their hosts by altering crab behavior to be less active in finding prey or to increase the reaction time of crabs to the presence of prey. Such behavioral changes can potentially reduce the functional response of the host by decreasing crab foraging time or lowering the frequency with which the crab encounters mussels.

In the present study, we measured the metabolic and digestive rates of infected and uninfected crabs in the laboratory to determine the influence of parasitic infection on these processes. We then conducted a series of observational studies to quantify crab activity levels and reaction time to the presence of mussels. Finally, we conducted a field survey to assess the microhabitat preferences of infected and uninfected crabs.

2. Materials and methods

2.1. Sampling

Eurypanopeus depressus were collected from oyster reefs within the North Inlet Estuary (33°20′N, 79°10′W, Georgetown, South Carolina) 24 h prior to experimentation. All oyster reefs were within 5 km of each other, and no oyster reef was closer than 200 m to another sampled reef. Infected crabs were identified by the presence of parasite externae, which signifies the parasite was mature (Alvarez et al., 1995). We only used infected crabs with a single mature externa to reduce any variation produced by multiple infections. Our sampling methods could not discern whether crabs were infected with the immature, internal phase of the parasite, so it is possible that some crabs categorized as "not infected" did have infections. We utilized male and female crabs in both infected and uninfected treatments because the effects of parasitic castration made distinguishing the sex of infected crabs difficult (Daugherty, 1969), and gender was not found to have an effect on any of the crab response variables (six separate generalized linear models $p > 0.28$). Therefore, crabs of both genders were grouped for the remainder of the analyses. We sampled and studied 160 infected and 160 uninfected crabs (carapace width 8–14 mm for both infected and uninfected crabs) between July 5 and August 15, 2013. Crabs were starved 24 h prior to experimentation to standardize hunger levels and were monitored for 24 h after their respective experiments to ensure none underwent ecdysis or extruded eggs. Each crab was used only once over the course of an experiment and no crabs were used in multiple experiments. Scorched mussels, *Brachidontes* exustus, are an important prey item of *E. depressus* (McDonald, 1982; Toscano et al., 2014) and were collected from the same reefs from which we sampled crabs (shell length 3.5–7.5 mm).

2.2. Metabolism

We determined whether parasitic infection reduced the metabolic rate of *E. depressus* by measuring the oxygen consumption rate of infected and uninfected *E. depressus* inside hermetically sealed individual plastic containers (20 × 12 × 11 cm). Filtered seawater (salinity 32–34 psu, temperature 24.3 ± 0.8 °C, 1 μm filter) was collected from North Inlet. Containers were set on a stirring plate and compartmentalized with plastic mesh in cylindrical form (diameter 12 cm, height 11 cm, pore size 0.5 cm). A magnetic stirrer was placed in one compartment to ensure all dissolved oxygen (DO) was distributed evenly within the container. The other compartment received either a single infected crab, uninfected crab, or no crab (control) depending on the treatment. Each trial had two replicates of each treatment, with five trials run over three consecutive days ($n = 10$ for each treatment). Trials lasted 1 h with a 10 min acclimation period beforehand to help reduce the effect of handling. Concentration of DO was measured every 10 min using a microprocessor dissolved oxygen meter with a Clark-type electrode (Hanna Instruments, HI 9146 N).

Individual crab metabolic rates were calculated by taking the average change in DO concentration between the 10 min intervals, standardized by container water volume and crab weight, then corrected for changes in oxygen consumption not attributable to crabs and/or the parasite by subtracting the average difference in DO consumption calculated from the control containers within the same trial. All statistical analyses were done in R, version 3.0.1 (R Development Core Team, Auckland, New Zealand). A Shapiro–Wilk test indicated that the data were not normally distributed. We therefore compared oxygen consumption between infected and uninfected crabs using a Wilcoxon rank sum test with continuity correction.

2.3. Digestion

We conducted observational studies to determine if reduced rates of mussel consumption reported for infected crabs could be attributable to slowed digestion rates, measured as gut passage time. Crabs collected from the North Inlet Estuary (carapace width = 9–13.5 mm) were starved for 24 h to ensure empty guts (Hill, 1976; Wolcott and Wolcott, 1987; personal observations) before being placed in individual cylindrical glass containers (height 5 cm, radius 3 cm) filled with seawater and allowed to acclimate for 5 min. After acclimation, a crushed mussel (shell length = 4–7 mm) was placed in the center of the container. We continuously observed the crabs for 1 h to determine when mussel consumption began and ended. After 1 h, crabs were checked every 10 min until they produced feces. Each trial had five infected and uninfected crabs with six trials run over six consecutive days ($n = 30$ for each treatment). Gut passage time was calculated as the time from initial food consumption to the time the crab first produced feces. We used a mixed-effects generalized linear model (GLM) with an exponential distribution to test the fixed effects of infection status and crab wet weight (g) as well as the random effect of trial on crab gut passage time. To determine if trial had a significant influence on