



What constrains the geographic and host range of the rhizocephalan *Loxothylacus texanus* in the wild?

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Abstract

In the Gulf of Mexico, the rhizocephalan barnacle *Loxothylacus texanus* parasitizes members of the genus *Callinectes*. In the past, research has primarily focused on the interaction between *L. texanus* and its blue crab host *Callinectes sapidus*. Only recently investigators have begun to examine the association that this parasite shares with other Gulf of Mexico hosts and little is known about interactions outside of the Gulf of Mexico. In the current study, settlement of *L. texanus* on a variety of potential hosts was examined. Settlement of *L. texanus* occurred not only on *C. sapidus* but also on the congener *Callinectes similis*, the grapsid *Sesarma cinereum* and the xanthid *Rhithropanopeus harrisi* collected from the Gulf of Mexico. *L. texanus* also settled on *C. sapidus* from Delaware Bay and *Callinectes ornatus* from Brazil. A carbohydrate-based cue appears to be the trigger for settlement in all cases. These results suggest that larval settlement may not play as important a role in determining host specificity as is often assumed. In fact, this type of nonspecific settlement may over evolutionary time lead to establishment of the parasite on new host species. Other factors such as physical barriers and immune response may aid in the control of such parasitic infections.

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1. Introduction

In the current age of fast ships and routine transatlantic voyages, introduced species have become an increasing problem in coastal marine ecosystems (Cohen and Carlton,

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1998). Ballast water taken on in a port city of one continent contains a myriad of larval species that are released into habitats free of natural predators on the other side of the ocean (Carlton and Geller, 1993). Carlton (1996) has estimated that everyday 3000 aquatic species are being transported around the globe.

A primary example of the effects of introduced species on native fauna is the European green crab, *Carcinus maenas*, which has had negative impacts upon salt marsh communities in New England, California, Canada, Australia, Tasmania and South Africa (Lafferty and Kuris, 1996). Lafferty and Kuris (1996) suggested that introducing the rhizocephalan, *Sacculina carcini*, which is a parasitic castrator of *C. maenas* in the Mediterranean Sea, might control the pest in San Francisco Bay. Information on rhizocephalan host specificity is limited, however, and the introduction of a parasitic rhizocephalan could be disastrous should local commercial species of crabs be susceptible as well. While some species have been shown to be fairly specific in their host preferences, others such as *S. carcini* are capable of infecting a broader range of species (Thresher et al., 2000). This lack of specificity is of concern to ecologists because these sorts of biological control agents are often considered as solutions in the control of non-indigenous species, but may have a dramatic impact on native species.

A good example of broad host recognition is seen in the rhizocephalan barnacle, *Loxothylacus texanus*. This species is known to parasitize crabs of the family Portunidae in the Gulf of Mexico including *Callinectes sapidus* (Adkins, 1972; Christmas, 1969; Harris and Ragan, 1970; Hochberg, 1988; More, 1969; Park, 1969; Ragan and Matherne, 1974; Wardle and Tirpak, 1991), *Callinectes marginatus* (Boschma, 1955), *Callinectes ornatus* (Park, 1969), and *Callinectes rathbunae* (Alvarez and Calderon, 1996).

Research on the rhizocephalan *L. texanus* has focused mainly on its interaction with the greater blue crab, *C. sapidus*, as this species is a valuable resource to both recreational and commercial fishermen along the Gulf Coast of the United States. It is only recently that investigators have begun to illuminate aspects of the association this parasite shares with other hosts (Alvarez and Calderon, 1996; Alvarez et al., 2002; Robles et al., 2002). Although the greater blue crab is found in coastal waters along the Atlantic seaboard (Williams, 1984) of North and South America, the parasite currently is found only in the Gulf of Mexico (Alvarez and Calderon, 1996) and the southern Atlantic coast of the United States north to South Carolina (Eldridge and Waltz, 1977, as cited in Shields and Overstreet, in press). It is unclear what biological or physical mechanisms prevent the parasite from naturally expanding its range. In fact, very little is known about the mechanisms that are used by the parasite to locate and initiate the symbiotic association with the host.

Rhizocephalan barnacles are released from their hosts as lecithotrophic, sexually dimorphic naupliar larvae. After a period of 3 days at 25 °C, these nauplii molt to become cyprid larvae. The cyprids have approximately 3–4 days to find a vulnerable host upon which to settle. A female cyprid will settle on a soft-shell juvenile blue crab and metamorphose into a dart-like kentrogon larvae (Yanagimachi, 1961; Høeg, 1985). After 60–70 h, a vermigon larvae leaves the kentrogon and enters the host crab (Glenner and Høeg, 1995; Glenner et al., 2000). Presumably, at least two cues are necessary for successful parasitism: one that enables the female cyprid to detect a vulnerable individual of a suitable host species and a second that induces metamorphosis of the cyprids. These cues are likely to be associated with the exoskeleton of the host crabs.

Lockwood (1967) divides the exoskeleton of decapod crustaceans into four regions: epicuticle, exocuticle, endocuticle and membranous layer. The outer, epicuticle layer is composed of proteins, carbohydrates and lipids. The epicuticle is thought to be responsible for restricting the permeability of the cuticle. *L. texanus* settlement assays using *C. sapidus* indicate that a carbohydrate component of this epicuticle layer is being used by the parasite to identify host crabs (Boone et al., 2003). Although similarities certainly do exist between the compositions of marine crustacean exoskeletons, a degree of variation in proteins among species has been reported (O'Brien et al., 1991). Such differences could be utilized by the parasite to identify the suitable species of host.

In the work presented here, we have examined the specificity of larval settlement of the rhizocephalan *L. texanus* as well as the cues necessary for settlement on individual host species.

2. Methods

2.1. Collection and maintenance of animals

Parasitized crabs were obtained from the Gulf Coast Research Lab, Ocean Springs, MS; Gulf Specimens Marine Lab, Panama, FL, and the Discovery Hall Program, Dauphin Island Sea Lab, Dauphin Island, AL, USA. Crabs with externae that were about to release could be recognized by their dark brown mantle cavity and were isolated in separate, aerated 19 l buckets of filtered seawater (25‰). The larvae are nonfeeding and were maintained in aerated buckets until the cyprid stage was reached (O'Brien, 1999a).

Uninfected Gulf of Mexico decapods (*Callinectes similis*, *Rhithropanopeus harrisi*, *Clibanarius vittatus*, *Sesarma cinereum*, *Libinia emarginata*) were obtained from Dauphin Island and Fowl River, AL using dip nets, seines and crab traps. Parasitized and uninfected crabs were maintained in separate recirculating seawater systems (300–450 l each) as described in Boone et al. (2003). Newly molted crabs were removed from the seawater system and frozen in their soft-shell state at -20°C until needed.

Crabs from two regions not known to be affected by *L. texanus* were also collected for comparison purposes. Soft-shell *C. sapidus* from Delaware Bay, Lewes, DE and *C. ornatus* from Ubatuba Bay, Sao Paulo, Brazil were frozen and transported to the University of South Alabama.

2.2. Design of settlement experiments

All settlement assays were performed using 11 cm diameter glass dishes. Soft-shell exoskeletons (Stages A and B) (Drach, 1939), or pieces of soft-shell exoskeleton of equal size were added to each dish. Cyprid larvae in 200 ml of seawater were placed in each dish. Only cyprids that were within 24 h post metamorphosis were used. All experiments were carried out using five replicates unless otherwise indicated. Each container was covered with aluminum foil to minimize salinity changes due to evaporation. After a period of 3 days, settlement was measured by counting kentrogon larvae. Those larvae that were easily removed with gentle shaking of the tissue were

considered unattached. In order to account for the unknown sex ratio of each brood of larvae, all replicates in a given experiment were performed using the same batch of released larvae, with the assumption that ratios remained constant within a single batch. Given the difficulty of distinguishing unsettled female cyprids from their male counterparts which will not settle, counts were made as a percentage of kentrogons attached to a given carapace piece compared to the total number of attached kentrogons on all of the substrates in that bowl (Boone et al., 2003). In all experiments, only one species was compared to the control.

2.3. Settlement on *L. texanus* hosts collected from uninfected regions

Both *C. sapidus* and *C. ornatus* are known to be infected by *L. texanus* in the Gulf of Mexico (Alvarez and Calderon, 1996). There are no reports of *C. sapidus* from the Atlantic coast of the United States, north of South Carolina, nor *C. ornatus* from South America south of the Amazon River outlet, being infected by the parasite. Crabs from these regions were transported to the University of South Alabama to compare settlement induction rates with that of *C. sapidus* from the Gulf of Mexico.

Soft-shell greater blue crabs were collected in Delaware Bay, Lewes, DE. *C. ornatus* (Portunidae) were collected from Ubatuba Bay, Sao Paulo, Brazil, a site where sacculinids have not been found on portunids (Mantelatto et al., 2003). Samples were initially held on ice at the site of collection and subsequently transported to the University of South Alabama under dry ice. The exoskeleton was removed and cut into strips as previously described and placed in glass fingerbowls containing cyprids (Boone et al., 2003). Blue crabs from the Gulf of Mexico were used as controls.

2.4. Species specificity

To analyze host specificity of the cyprid larvae, five species of decapods found in the Gulf of Mexico were compared to *C. sapidus* in separate experiments. Exoskeleton from the different species of soft-shell crabs was removed as previously described for *C. sapidus* and placed in glass finger bowls with equivalent sized pieces of exoskeleton from *C. sapidus* as controls. The species of crabs used were as follows: lesser blue crab: *C. similis* (Brachyura: Portunidae), white fingered mud crab: *R. harrisii* (Brachyura: Xanthidae), striped hermit crab: *C. vittatus* (Anomura: Diogenidae), marsh crab: *S. cinereum* (Brachyura: Grapsidae) and nine-spined spider crab: *L. emarginata* (Brachyura: Majidae). Each species was tested in a separate experiment.

2.5. Determination of basic chemical cues using other species

Exoskeleton strips with proteins, lipids or carbohydrates removed were placed together in a test dish along with an untreated postmolt strip. Three species, *C. ornatus*, *C. similis* and *S. cinereum*, were tested in separate experiments. Carbohydrates were removed using 10 mM sodium periodate in 50 mM sodium acetate (pH 4.5) followed by 1 M sodium borohydride in Tris-buffered saline. Lipids were removed using chloroform and proteins

were removed using Proteinase K (6 Anson units/ml U.S. Biochemical Cat. No 20818 in 10 mM Tris, pH 7.8) (Boone et al., 2003).

2.6. Statistical analysis

It was not feasible to control the exact number of kentrogons added to each bowl. Hence, the response variable for each treatment and replication was the number of kentrogons observed on a given substrate in a bowl, compared to the total number of kentrogons on all substrates in that bowl. For purposes of statistical analyses, the response variable was then expressed as a percent.

All statistical tests were considered significant at $p < 0.05$. Each experiment was analyzed as a Randomized Block Design (Zar, 1996). Under this design, the bowl was considered the block (random effect) and the exoskeleton substrate strips the main (fixed) effect (Model III). Given that percentages follow a binomial distribution (Sokal and Rohlf, 1981), all values of the response variable were arcsin transformed before the ANOVA (Zar, 1996). The Tukey multiple comparison test was then performed on all statistically significant ANOVAs (Zar, 1996). The family error rate of these comparisons was set at $p < 0.05$, with individual errors dependent on the number of comparisons to be made. Data in the text for each treatment are reported as mean percent settled $\pm 95\%$ confidence interval. Confidence intervals were based on the pool error term from the ANOVA.

3. Results

3.1. Settlement on *C. sapidus* and *C. ornatus* collected from uninfected regions

No significant difference in settlement was observed between blue crabs collected from Delaware Bay, DE ($48.0 \pm 16.9\%$) and Dauphin Island, AL ($51.9 \pm 16.9\%$). Likewise, no significant difference in settlement was observed when *C. ornatus* ($42.1 \pm 10.7\%$) was compared to *C. sapidus* ($57.9 \pm 10.7\%$). Removal of carbohydrates from the exoskeleton of *C. ornatus* resulted in decreased settlement ($p < 0.001$, Tukey) (Fig. 1); however, an increase in settlement was not observed following the removal of lipids. Lipid- and protein-removed strips did not differ significantly from the soft-shell control ($p = 0.8030$ and $p = 0.6373$, respectively, Tukey).

3.2. Settlement on other species of decapods from the Gulf of Mexico

Settlement occurred on three species of crabs including *C. similis*, *R. harrisii* and *S. cinereum*. There was no significant difference between settlement on *C. sapidus* and *C. similis* ($42.1 \pm 7.2\%$ vs. $57.9 \pm 7.2\%$ on *C. sapidus*), nor between *C. sapidus* and *S. cinereum* ($68.8 \pm 14.2\%$ vs. $31.1 \pm 14.2\%$ on *C. sapidus*). Settlement on *R. harrisii* ($14.0 \pm 3.2\%$) was, however, significantly less than that of *C. sapidus* ($86.0 \pm 3.2\%$) ($p < 0.001$, Tukey). No settlement was observed on *C. vittatus* nor *L. emarginata*.

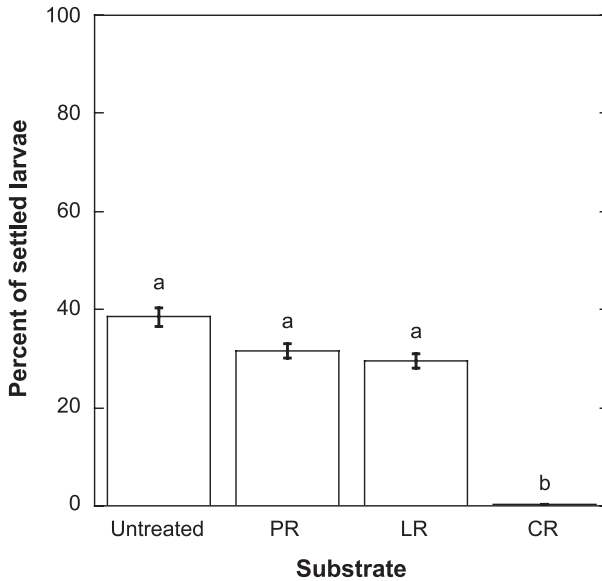


Fig. 1. Chemical cues of *C. ornatus*: Percent settlement on postmolt epicuticle following the removal of proteins (PR), lipids (LR) and carbohydrates (CR) as compared to untreated epicuticle. Settlement percentages with the same letters above error bars (within this figure) were not significantly different at $p > 0.05$. Error bars indicate 95% confidence intervals.

3.3. Chemical cues on other Gulf of Mexico species

Cyprid settlement on the grapsid crab, *S. cinereum*, followed a similar pattern to that on *C. sapidus* (Fig. 2), with removal of carbohydrates causing a significant decrease in settlement ($p < 0.001$, Tukey). No difference in settlement was seen however with removal of either proteins ($p = 0.9271$, Tukey) or lipids ($p = 0.4109$, Tukey). Removal experiments using exoskeleton from *C. similis* (Fig. 3), however, indicated that the absence of proteins significantly enhanced settlement ($p < 0.001$, Tukey) whereas a significant decrease was still observed with carbohydrate removal ($p < 0.001$, Tukey). Lipid removal did not result in a significant difference in settlement in this case ($p = 0.0612$, Tukey).

4. Discussion

While the exact composition of the settlement cue remains unknown, it does not appear to be specific to the greater blue crab, *C. sapidus*. The consistency of the carbohydrate-based cue across multiple species is significant. The lack of “lipid-removed” enhanced settlement in other species as well as the “protein-removed” enhancement seen in *C. similis* suggest that while mannose is a significant component of the recognition signal (Boone et al., 2003), this is an intricate settlement system that probably involves more than one carbohydrate moiety.

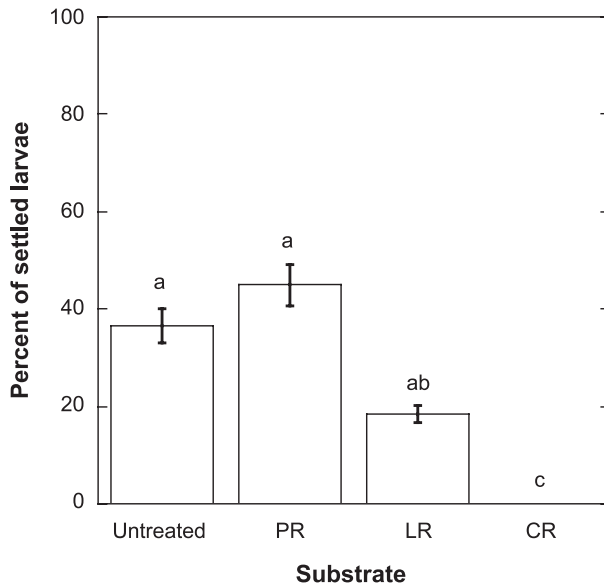


Fig. 2. Chemical cues of *S. cinereum*: Percent settlement on postmolt epicuticle following the removal of proteins (PR), lipids (LR) and carbohydrates (CR) as compared to untreated epicuticle. Settlement percentages with the same letters above error bars (within this figure) were not significantly different at $p > 0.05$. Error bars indicate 95% confidence intervals.

Of the five Gulf of Mexico decapod species tested, soft-shell carapace from three crabs, the lesser blue crab *C. similis*, the grapsid *S. cinereum* and the xanthid *R. harrisii* were able to induce cyprid metamorphosis into kentrogons. While *C. sapidus* and *C. similis* are congeners, *R. harrisii* and *S. cinereum* belong to families other than the Portunidae. None of these species are known to be infected by *L. texanus*. *R. harrisii*, however, is parasitized by a congener of *L. texanus*, *Loxothylacus panopaei*, as are other xanthids such as *Panopeus herbstii*, *Eurypanopeus depressus* and *Tetraxanthus rathbunae* (Boschma, 1928; Reinhard and Reichman, 1958; Van Engle et al., 1966; Daugherty, 1969). Perhaps the receptors of the two congeners are evolutionarily conserved. It would be interesting to test whether cyprids of *L. panopaei* would settle upon portunid exoskeleton and, if so, whether the immune response of a natural host could destroy the congener of its parasite. Settlement on *S. cinereum* was unexpected for the initial intention was to use it as a negative control. This species is adapted to exposed intertidal habitats and on humid evenings in Mobile, AL can often be found in lawns and around houses 20–30 m from the water (O'Brien, personal observation). Certainly any cyprid or kentrogon that settled upon this species would be subjected to desiccation. One could argue that the semi-terrestrial lifestyle of grapsid crabs would decrease their vulnerability to marine parasites such as *L. texanus*, yet grapsids return to the water to molt and some species do harbor sacculinid rhizocephalans (Boschma, 1955; Hartnoll, 1967). In similar studies using *S. carcini*, the parasite barnacle of *C. maenas* (Portunidae), settlement was observed on the grapsid crab, *Paragrapsus gaimardii*, in both laboratory and field experiments although this exposure

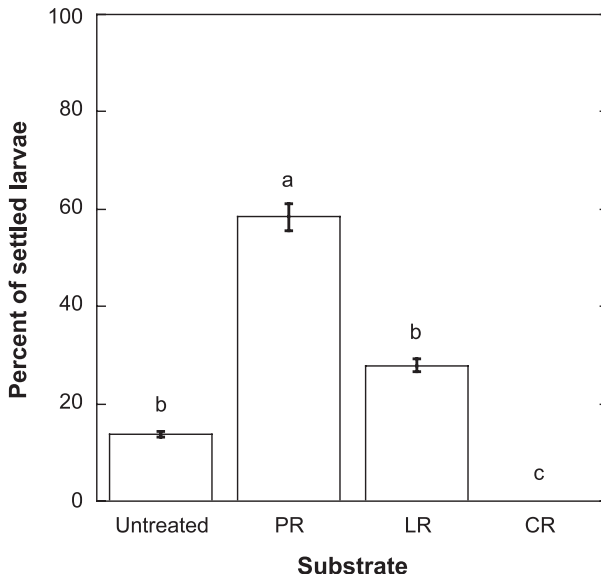


Fig. 3. Chemical cues of *C. similis*: Percent settlement on postmolt epicuticle following the removal of proteins (PR), lipids (LR) and carbohydrates (CR) as compared to untreated epicuticle. Settlement percentages with the same letters above error bars (within this figure) were not significantly different at $p > 0.05$. Error bars indicate 95% confidence intervals.

did not ultimately lead to infection (Thresher et al., 2000). There are many rhizocephalan species that occur on more than one species of host (Boschma, 1962; Høeg, 1982; Hoggarth, 1990; Ritchie and Høeg, 1981) although specificity commonly occurs at the host family level (Boschma, 1962). Hence, it seems that larval settlement may not play as important a role in determining host specificity as one might assume and that over an evolutionary time scale, these normally “fatal” errors may actually lead to successful establishment on a new host species (Høeg, 1995). Clearly parasite settlement is not to be equated with parasitic infection, and the data reported here suggest that physical factors (such as the ability of the vermigon to penetrate host cuticle) or biological factors (such as the ability of the interna to escape the immune defenses of the host) may play a very important role in determining infection success. Since the parasites cause reproductive death, one would expect there to be strong selective pressure for host defense mechanisms, mechanisms successful parasites circumvent prior to infection (see O’Brien, 1999b for a discussion of The Red Queen Hypothesis and how it is related to interactions between parasitic castrators and their hosts).

Our data indicate that physical barriers may be primarily responsible for the limited range of this rhizocephalan species. The greater blue crab can be found all along the eastern seaboard of North and South America but it is only infected by *L. texanus* south of South Carolina throughout the Gulf of Mexico. Likewise, the portunid, *C. ornatus* whose range extends continuously from the Gulf coast to the tip of South America, is only known to be infected in the Gulf of Mexico. The absence of the parasite from regions of the host

range could be caused by either biological barriers (i.e. an effective immune response) or physical barriers (i.e. freshwater outflow from the Amazon River). Reisser and Forward (1991) determined that *L. panopaei* did not survive in low salinities and O'Brien et al. (1993a,b) and Tindle et al. (2004) found similar patterns with larvae of *L. texanus*. These data suggest that areas of low salinity such as would be found in upper regions of bays and estuaries could serve as refuges for crabs from the rhizocephalans. In this study, settlement assays using *C. sapidus* from Delaware Bay, DE revealed no difference in kentron numbers as compared to controls. Similarly, *C. ornatus* collected from the coast of Brazil were able to induce cyprid settlement. These results provide evidence that unless crabs in these regions possess a more effective immune response to the parasite, the absence of *L. texanus* from areas of its host ranges is more likely to be a result of a physical rather than a biological barrier.

5. Conclusions

From this work, it is clear that settlement and potentially infection could occur on a number of host species if the parasite is present in the habitat. In other words, settlement does not appear to be particularly species specific. Clearly until a better understanding of the infection process is developed, the introduction of sacculinids to new habitats in an effort to control introduced pests is a risky proposition.

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