

Host Selection, Chemical Detection, and Protection of the Symbiotic Pinnotherid Crabs *Dissodactylus crinitichelis* and *Clypeasterophilus rugatus* Associated with Echinoderms

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Abstract

Host selection by *Dissodactylus crinitichelis* and *Clypeasterophilus rugatus* for different echinoderms was investigated. Initial preference (within 24 h of collection) and preference after "conditioning" with an alternate host were compared. *D. crinitichelis* initially preferred its field host, the sand dollar *Encope michelini*, but after conditioning switched its host preference to a non-field host, the sea biscuit *Clypeaster rosaceus*. This switch in host preference after conditioning occurred despite consumption of numerous crabs (86 out of 167) by *C. rosaceus*. *Clypeasterophilus rugatus* initially preferred its field host, the sea biscuit *Clypeaster rosaceus*, but showed little change in host preference after conditioning. These results indicate significant behavioral differences in these closely related crabs in their association with echinoderms. Chemical detection by the crabs was investigated, and no significant use of chemical cues by adults or juveniles of either species was found, even though chemical responses have been observed in other closely related pinnotherid crabs. Using the checkered pufferfish, *Sphoeroides testudineus*, as a predator, the possibility that crabs may receive protection by living with echinoderms was also examined. Crabs with echinoderms survived significantly longer than crabs without echinoderms, which is the first direct evidence that these crabs are protected by associating with echinoderms.

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1. Introduction

Many examples of symbiosis exist in the sea where smaller symbionts or epibionts seek out larger hosts, presumably because suitable refuge is limited and competition for space high. Such associations allow for the study of behavioral interactions among species and innovations by the symbionts to facilitate the initiation and maintenance of the association. A common marine example involves pinnotherid crabs of the genera *Dissodactylus* and *Clypeasterophilus* living with various types of echinoderms. A specific example involves *Dissodactylus mellitae*, which lives on the sand dollar *Mellita quinquesperforata* (in Beaufort, North Carolina) clinging to spines via modified chelae and pereopods (Telford, 1982; Bell, 1987). Because of their small size and inconspicuous nature, it is unlikely the crabs provide benefit such as protection to the echinoderms. In fact, they are generally regarded as commensals or parasites (Telford, 1982). How the crabs benefit from the symbiosis has been a topic of investigation. Some reports indicate that *D. mellitae* eats spines and tube feet (Telford, 1982; Bell, 1987), as these structures have been found in gut analyses (Telford, 1982). However, it is unclear whether the tissues consumed were live or necrotic; thus, the relative harm to the echinoderm is unknown. The crabs also frequent the oral surface of the sand dollar (Bell, 1987). While near the mouth or ambulacral grooves, food collected by *M. quinquesperforata* may also be available to the crabs. Although echinoderms are unlikely to be effectively defended by these tiny crabs, protection to the crab is a possible benefit of the association. The crab's cryptic coloration and positioning on the oral surface of the sand dollar may provide protection from predators. No studies have tested this protection hypothesis for any of the pinnotherid crabs living with echinoderms. Interestingly, injured sand dollars produce an injury product that is unattractive to *D. mellitae* causing them to avoid settlement on the injured echinoderm (Gray et al., 1968). It may be that attraction of scavengers or echinoderm predators makes the host less "attractive" as a refuge for protection. Additionally, the injury to and possible demise of the host echinoderm may signal a dispersal response by the crabs to find a new host.

Several studies have examined the use of chemical cues by symbiotic pinnotherid crabs in locating hosts (Johnson, 1952; Davenport et al., 1960; Gray

et al., 1968; Derby and Atema, 1980; Stevens, 1990; Brooks and Rittschof, 1995). A "host factor" or chemical cue is used by *D. mellitae* to locate its echinoderm host (Gray et al., 1968). Bell (1987) stated that *D. mellitae* has been found on three different sand dollar hosts (*Mellita quinquiesperforata*, *Echinarachnius parma*, *Encope michelini*) within its range (Massachusetts to Florida). Gray et al. (1968) discovered that *D. mellitae* could be induced in the laboratory to switch its chemical response from its North Carolina host, *Mellita quinquiesperforata*, to another species of sand dollar, *Encope michelini*, after 1–2 days of acclimation to *E. michelini* in the absence of *M. quinquiesperforata*.

A second species of crab found living with an echinoderm is *Clypeasterophilus rugatus* (formerly *Dissodactylus rugatus* Bouvier, 1917, a senior synonym of *D. calmani* Rathburn, 1918). The genus *Clypeasterophilus* was coined to encompass four former *Dissodactylus* spp. based on morphological criteria and their propensity for symbioses with irregular echinoids of the genus *Clypeaster* (Campos and Griffith, 1990). *Clypeasterophilus rugatus* has been found from Florida to the southern Caribbean Sea (Werding and Sanchez, 1989). *Clypeasterophilus rugatus* has been found occasionally free-living in the Florida Keys (Voss and Voss, 1955); however, it is typically found on the sea biscuit *Clypeaster rosaceus*. Less research has been done on this species, but findings thus far suggest it is more specialized to life on its host than is *D. mellitae*. *Clypeasterophilus rugatus* differs by having a flatter, more quadrangular carapace with dark brown, white-banded legs. The host echinoderm, *Clypeaster rosaceus*, is also dark brown in color (Telford, 1982). Telford also found the claws of *Clypeasterophilus rugatus* are the least typical of the entire genus, with sharp edges specialized for excising soft tissues between the spines of *Clypeaster rosaceus*. Other species typically have spine-crushing/cutting claws. Telford (1982) also found that stomach contents of *Clypeasterophilus rugatus* were composed primarily of brown tissue presumably from *Clypeaster rosaceus*, whereas the other crab species consumed mostly host spines.

Another crab that lives on echinoderms is *Dissodactylus crinitichelis*. This species ranges from North Carolina (USA) to Brazil with one report off Colombia (Werding and Sanchez, 1989). *D. crinitichelis* has been found on five different hosts (four of which are sand dollars: *Meoma ventricosa*, *Encope michelini*, *Encope emarginata*, *Leodia sexiesperforata*; and the fifth is a burrowing sea biscuit, *Clypeaster subdepressus*) in different parts of its range (Werding and Sanchez, 1989). Like *D. mellitae*, the behavioral plasticity of *D. crinitichelis* may provide the crab increased opportunities for alternate hosts should populations of its normal echinoderm host decline or become less suitable. The coloration of *D. crinitichelis* is creamish white with irregular translucent patches on the carapace. This coloration appears to blend well with the sand often covering their host. Other characteristics of *D.*

crinitichelis are similar to those of *D. mellitae* such as their diet of host spines, similar morphological larval features, and living on burrowing echinoderm hosts. Telford (1978) noted that *D. crinitichelis* occasionally left its host and moved to another host if conditions such as crowding occurred.

No reports have examined the role of chemical cues used by adults (or juveniles) of either *D. crinitichelis* or *C. rugatus* in locating their hosts. Recent studies have focused on the larval characteristics of these species (Pohle and Telford, 1981; Pohle, 1984). Interestingly, Pohle (1984), while investigating larval characteristics, found that all three species (*D. mellitae*, *D. crinitichelis*, and *C. rugatus*) required exposure to their hosts before they could metamorphose into the first crab instar. This finding suggests these crabs are obligate symbionts for at least part of their life history.

In the present study, we examined three major topics: 1) host selection, by comparing the initial host preferences of *C. rugatus* and *D. crinitichelis* for echinoderm hosts, determining if these preferences can be modified by conditioning these crabs with echinoderms other than their field host, and comparing the findings to previous studies of a closely related crab, *D. mellitae*; 2) chemical detection, by testing the response of crabs to odors from their echinoderm host(s); and 3) crab protection, by measuring crab survival time with and without the echinoderm as well as with an artificial host in the presence of a fish predator.

2. Materials and Methods

Collection and maintenance of specimens

Crabs, sea biscuits, sand dollars, sea urchins, and fish (predators) were collected from the southeast coast of Florida (several sites off Palm Beach, Broward and Dade counties) at depths ranging from 1–10 m from May 1999 through April 2000. Care was taken during collection of the symbionts to prevent separation of the ectosymbiotic crabs from their echinoderm host by placing the symbionts together in resealable plastic bags upon collection. Sea urchins (*Lytechinus variegatus*) were also collected and placed in resealable plastic bags. Pufferfish were captured using a seine net.

All animals were kept in seawater (34–38‰) while in transit from the collecting sites to the laboratory at Florida Atlantic University. In the laboratory, the animals were maintained in 10 l (40 × 21 cm at the base) saltwater aquaria (34–38‰) each equipped with a re-circulating water filter apparatus and exposed to a 12L:12D photoperiod at 25–28°C. All animals were fed Tetramin flake fish food weekly. Animals were not fed within 24 h of trials.

Habitat description and field associations

The echinoderms were all found off the SE Florida, yet their specific habitats differed. *Encope michelini* was found in open sandy areas approximately 6–10 m deep. The current in these areas was fairly strong as was evident by the rippled sandy bottom. A thin layer of sand (particle size = <1 mm) covered these sand dollars. *E. michelini* typically cover themselves with sand by burrowing just beneath the sea bottom (Hendler et al., 1995). The dark brown or deep purple coloration of the sand dollar was completely masked by the sand. While collecting, we noticed that the sand dollars leave trails in the sand as they burrow. The trails (approx. 0.25–1.0 m long) led directly to the sand dollars. They were typically found within 0.5–1.0 m from each other. *E. michelini* is flat with one slit in its test, has a short carpet of spines, and feeds on particulate matter in the substrate of the sea bottom (Hendler et al., 1995). It has been found from North Carolina to the southern tip of Florida and in the Gulf of Mexico. *Dissodactylus crinitichelis* was only found with *E. michelini*, and individuals were typically located on the sand dollars' oral surfaces. Though we did not obtain a count of crabs per host or monitor sex differences for this study, we typically found hosts housing two to four adult crabs, up to 20 juvenile crabs, or a combination of adult (<3) and juvenile crabs (<10).

Clypeaster rosaceus was found in or near *Thalassia testudinum* beds in water approximately 1–3 m deep and calmer than that of the sand dollar. These sea biscuits do not burrow but were instead covered by dead *Thalassia* blades and rubble (average particle size = 5–7 mm). This behavior is typical of *Clypeaster rosaceus* (Hendler et al., 1995). The sea biscuit appears inflated compared to the flat *E. michelini*. It is dark brown in color with spines that are relatively longer and more rigid than that of *E. michelini*. *Clypeaster rosaceus* has been found from South Carolina to south Florida, in the Gulf of Mexico, and along the coast of South America (Hendler et al., 1995). They are found both in aggregations and up to several meters from other *C. rosaceus*. Their diet consists of algae and seagrass. *Clypeasterophilus rugatus* was only found with *Clypeaster rosaceus*. Individual crabs were typically found on the oral surface of the host but were occasionally found under debris on the aboral surface. We observed a maximum of ten crabs per host with a typical amount being two to three adults, three to six juveniles per host, or a combination of adult (<3) and juvenile crabs (<7).

Crab identification

Crab species within the *Dissodactylus* complex (including those in the genus *Clypeasterophilus*) were identified by morphological characteristics (e.g., coloration, spine length, and size). Because of their small size (maximum

carapace width in this study was 10 mm), several sources were used to identify the crabs (Rathburn, 1918; Gruner, 1973; Werding and Sanchez, 1989), as well as having the crabs' identities verified by Dr. Darryl Felder of the University of Louisiana-Lafayette and the Smithsonian's laboratory in Fort Pierce, Florida.

Host preference trials

Initial preference tests – Trials were done within 24 h of collection of the crabs to determine their preference for echinoderm hosts. Each crab was offered a choice between two echinoderms: the normal echinoderm host (i.e., its field-collected host) and another echinoderm known to be sympatric off the SE Florida coast. The following echinoderm pairings were offered: 1) *Clypeaster rosaceus* vs. *Encope michelini*; 2) *Clypeaster rosaceus* vs. *Lytechinus variegatus*; and 3) *Encope michelini* vs. *Lytechinus variegatus*. The two echinoderms were placed at opposite ends of a 7.5 l (30 × 15 cm at the base) aquarium. A single crab was placed into the aquarium halfway between the two echinoderms. The position of the crab was recorded after 1 h, and if the crab was touching an echinoderm at the end of 1 h, a choice was recorded.

Initial preference trials were done in both light and dark conditions to control for possible light/observer effects. Crab size was also recorded during the initial preference trials to determine its influence on host preference.

Post-conditioning preference tests – Only those crabs that initially chose their normal field host were used in the conditioning trials, to establish a baseline from which host preference change could be detected. To begin the conditioning, each crab was placed in an aquarium with the echinoderm not chosen in the initial preference test. Sea urchins were eliminated from further trials after observing their tendency to consume crabs during the initial preference trials. Therefore, the hosts used in the conditioning trials included *Clypeaster rosaceus* and *Encope michelini*. Crabs were observed daily to determine if contact with the "substitute" echinoderm was established and maintained. Preference tests were then repeated for each crab at one-week intervals for up to 4 weeks, using the protocol after Gwaltney and Brooks (1994). Each crab was removed from its "conditioning" aquarium and placed in another aquarium for 1 h where it was given a choice between the same echinoderms offered in the initial preference tests (excluding the sea urchin *Lytechinus variegatus*). Crabs that again chose their "initially-preferred" field host were returned to the "conditioning" aquarium for another one-week interval. Data for all preference trials were analyzed using the binomial (Z) test.

Chemical detection trials

Chemical detection experiments were done in a clear, Plexiglas, four-

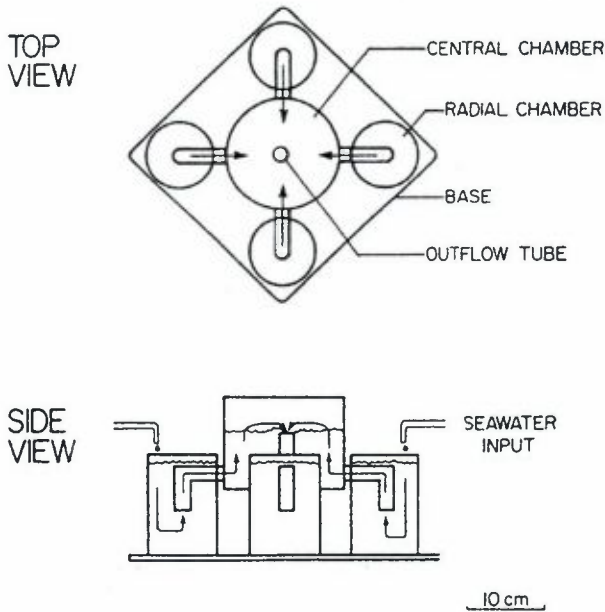


Figure 1. Four-chambered choice apparatus used in chemical detection tests. For size reference, the square base of the apparatus was 25.5 cm in length on each side.

chambered choice apparatus (Fig. 1, after Derby and Atema, 1980; Stevens, 1990; Brooks and Rittschof, 1995) under constant dark conditions at 24–28°C. A group of conspecific crabs (26–48 individuals) was placed randomly in the central chamber, and the number entering each radial chamber after 10 h was recorded. Each species of crab was tested separately, and each crab was tested once. In controls, all four radial chambers received aged seawater (held at least seven days in sealed holding tanks) at a rate of 10 ml/min (this rate is comparable to drip rates used in previous studies: Derby and Atema, 1980; Brooks and Rittschof, 1995). In each treatment, water from a container holding 2–3 individuals of a single echinoderm species dripped (10 ml/min) into one of the four chambers. The other three chambers received aged seawater as in the control. The echinoderm species used in each trial was the normal field host for the crab species being tested. Between treatments, the choice apparatus was rinsed with hot tap water followed by seawater. Also, the chamber that received water from the echinoderm container was assigned randomly. Results of the chemical detection trials were analyzed by comparing the number of crabs entering each radial chamber to the expected probability of 25% using the χ^2 test (Ambrose and Ambrose, 1987).

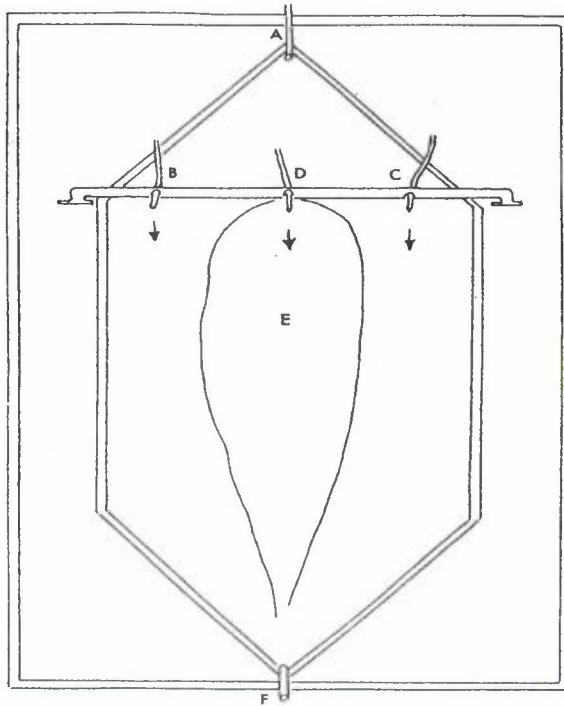


Figure 2. Trough apparatus used in chemical detection tests. The apparatus consisted of a 35.5×61.0 cm sheet of 1.1 cm Plexiglas, from which a 25.4×50.8 cm hexagonal center had been removed, cemented to a whole 35.5×61.0 cm sheet of 0.3 cm Plexiglas creating a shallow trough. A,B,C = carried aged seawater; D = carried host water; E = critical area; F = outlet.

The chemical detection response of the crabs was also tested using a Plexiglas, trough apparatus (Fig. 2, after Gray et al., 1968) under constant dark conditions at 25–28°C. Crabs were tested one at a time by placing the crab randomly into the trough. Aged seawater entered one end of the trough and flowed out the opposite end. Aged seawater from the two outer tubes and concentrated host water (host held in seawater for 24 h prior to testing) dripped into the trough at equal rates. The crab's proximity to the critical area (in the center of the trough) was noted 20 min after being placed into the trough and compared to a random distribution within the trough.

Predation trials

Sphoeroides testudineus (checkered pufferfish) was used in these trials to

determine the possibility that crabs may receive protection as a result of associating with echinoderms. This fish species is sympatric with the echinoderms and crabs used in this study. Trials were performed in 7.5 l (30 × 15 cm at the base) aquaria with a thin layer of sand on the bottom. For each crab species, a single crab was added to the aquarium in the controls and a single crab with an original host echinoderm in the treatments. Crabs in aquaria with the echinoderm present were allowed to associate with the host 24 h prior to testing. One fish was added to each aquarium. Survival time was recorded at 30 min intervals for 180 min. A blind (with small holes to allow for periodic observation) was constructed from cardboard to minimize observer interference. Average survival times for each crab species with and without an echinoderm were calculated and analyzed using the Wilcoxon Signed Rank test or the paired t-test, depending on whether the data passed the assumptions of the statistical test (Sigmastat 2.03).

Substitute host – Trials using a black jar lid (diameter = 9.5 cm and height = 1.5 cm) as a substitute host were conducted with each crab species to determine whether the crabs could receive protection by positioning themselves near an inanimate, dark object. The experimental procedure and analyses were identical to those described above with a living echinoderm host.

3. Results

General observations

In the laboratory, *Dissodactylus crinitichelis* was seen occasionally off its echinoderm host, though never venturing more than 2–3 cm away. The crabs were usually on the oral surface of the sand dollar. In the laboratory, *Clypeasterophilus rugatus* was most often found on the sea biscuit's oral surface though occasionally crabs were found either crawling on the echinoderm's aboral surface or on the aquarium bottom 1–3 cm from the host. During conditioning trials, each crab species maintained similar behavior with the exception of the larger *C. rugatus* crabs having difficulty getting beneath the sand dollar. These large crabs often remained on the perimeter of the sand dollar.

Host preference trials

Initial preference tests – Table 1 summarizes the results for these tests. *Dissodactylus crinitichelis* collected from *Encope michelini* (sand dollar) in the field showed a significant preference for this echinoderm over *Clypeaster rosaceus* (sea biscuit) and *Lytechinus variegatus* (sea urchin).

Table 1. Results of initial preference tests made within 24 h of collection. Each crab was given a choice between an echinoderm of the species from which it was collected and another sympatric echinoderm species found off the SE Florida coast. Results of all choice tests were statistically significant ($p < 0.001$ for all Z tests).

	N	Sand dollar	Sea biscuit	Sea urchin	Z
<i>Dissodactylus crinitichelis</i>					
Sand dollar versus sea biscuit	226	167 (74%)	59 (26%)		10.11
Sand dollar versus sea urchin	15	14 (93%)		1 (7%)	4.35
Sea biscuit versus sea urchin	11		11 (100%)	0	4.26
<i>Clypeasterophilus rugatus</i>					
Sand dollar versus sea biscuit	97	12 (12%)	85 (88%)		10.44
Sand dollar versus sea urchin	13	13 (100%)		0	4.71
Sea biscuit versus sea urchin	16		16 (100%)	0	5.30

Table 2. Results of initial host preference versus crab size. "<5 mm" denotes those crabs with a carapace width of less than 5 mm. ">5 mm" denotes those crabs with a carapace width of equal to or greater than 5 mm. No significant differences in preference for either size class were observed for either crab species.

	N	Sand dollar	Sea biscuit	Z
<i>Dissodactylus crinitichelis</i>				
<5 mm	69	56 (81%)	13 (19%)	0.27 ($p > 0.05$)
>5 mm	20	15 (75%)	5 (25%)	
<i>Clypeasterophilus rugatus</i>				
<5 mm	27	3 (11%)	24 (89%)	1.01 ($p > 0.05$)
>5 mm	14	4 (29%)	10 (71%)	

Clypeasterophilus rugatus collected from *C. rosaceus* in the field showed a significant preference for this echinoderm over *E. michelini* and *L. variegatus*. Both crab species showed a significant preference for *C. rosaceus* and *E. michelini* over *L. variegatus*.

The control tests run under dark conditions were not significantly different from the tests run under light conditions, specifically, 88 (or 74%) *Dissodactylus crinitichelis* chose *Encope michelini* ($p = 0.592$) and 24 (or 89%)

Table 3. Results of post-conditioning preference tests, comparing initial host preferences with subsequent preferences after "conditioning" (final preference). "Deaths during conditioning" = crabs that died during the conditioning periods. *D. crinitichelis* switched its preference after conditioning to the sea biscuit. All choice tests were statistically significant ($p < 0.001$ for all Z tests).

	N	Sand dollar	Sea biscuit	Z	Deaths during conditioning
<i>Dissodactylus crinitichelis</i>					
Sand dollar versus sea biscuit	81	17 (21%)	64 (79%)	7.23	86 of 167 (51%)
<i>Clypeasterophilus rugatus</i>					
Sand dollar versus sea biscuit	81	17 (21%)	64 (79%)	7.23	4 of 85 (5%)

Clypeasterophilus rugatus chose *Clypeaster rosaceus* ($p=0.469$) under dark conditions. Therefore, all further preference tests were performed under light conditions.

Because of the lack of preference by the crabs for *Lytechinus variegatus*, and observations that *L. variegatus* readily consumed many crabs, this echinoderm was no longer used as a host in any further trials.

Crab size influence – The influence of crab size on initial host preference was examined for some of the crabs. The crab sizes were divided into two categories, <5 mm and >5 mm. Both sizes of both crab species initially preferred their field host ($p < 0.001$). Table 2 shows there were no significant differences in initial host preference between the two size categories of *Dissodactylus crinitichelis* ($p=0.786$) or between the two size categories of *Clypeasterophilus rugatus* ($p=0.311$).

Post-conditioning preferences – Most (74%) *Dissodactylus crinitichelis* initially preferred *Encope michelini*, but switched their preference after conditioning to 79% choosing their "conditioning" host, *Clypeaster rosaceus*. Most (88%) *Clypeasterophilus rugatus* initially preferred *Clypeaster rosaceus*, and after conditioning 79% still preferred *C. rosaceus* (see Table 3).

Crab attrition – Significant crab attrition (deaths) occurred in some of the conditioning trials. *Dissodactylus crinitichelis* had the highest number of deaths (86 out of 167 crabs). *Clypeaster rosaceus* likely consumed these crabs over the four weeks of conditioning as no carcasses were found in the conditioning aquaria. *Clypeasterophilus rugatus* had far less attrition (4 out of 85 crabs).

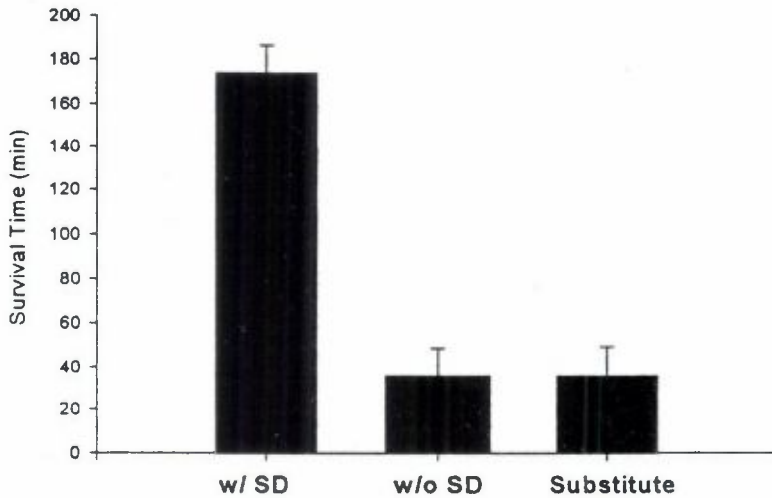


Figure 3. Predation results showing survival times for *D. crinitichelis* with the pufferfish predator *Sphoeroides testudineus*. SD = sand dollar *Encope michelini*. Control = without sand dollar (n=10); treatments = with sand dollar (n=10) OR with substitute host (black jar lid)(n=5). Bars represent standard deviation. Survival times were significantly longer with the SD than without ($p=0.002$, Wilcoxon Signed Rank Test). Small sample size for the substitute host precluded statistical analysis.

Chemical detection trials

Based on the trials using the 4-chamber choice apparatus, there appeared to be no significant chemical detection response from either crab species. The number of crabs entering the chambers did not differ significantly from random using the chi-square test (Ambrose and Ambrose, 1987). Trials using the trough apparatus also revealed no significant data.

Predation trials

Ten controls (no echinoderm) and ten treatments (echinoderm present) were run with the pufferfish (predator) and each crab species. Eight out of ten *Dissodactylus crinitichelis* with *Encope michelini* survived 180 min, but all ten without the echinoderm were eaten by the pufferfish within the first 30 min ($p=0.002$) (see Fig. 3). All ten *Clypeasterophilus rugatus* with *Clypeaster rosaceus* survived 180 min with the fish, while the ten crabs without the echinoderm were all eaten within the first 60 min ($p=0.002$) (see Fig. 4).

Substitute host – Because of low availability of *Dissodactylus crinitichelis*,

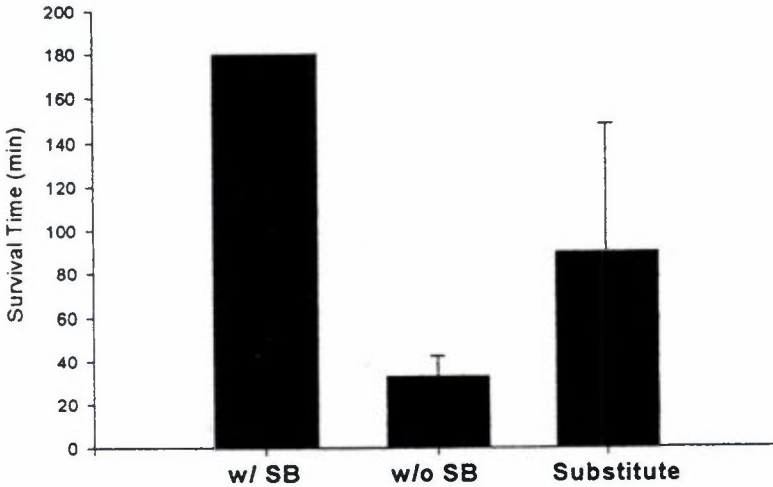


Figure 4. Predation results showing survival times for *C. rugatus* with the pufferfish predator *Spherooides testudineus*. SB = sea biscuit *Clypeaster rosaceus*. Control = without sea biscuit (n=10); treatments = with sea biscuit (n=10) OR with substitute host (black jar lid)(n=10). Bars represent standard deviation. Survival times were significantly longer with the SB than without ($p=0.002$, Wilcoxon Signed Rank Test). Survival times with the substitute host were significantly higher than the control ($p=0.008$), but still less than survival times with the SB ($p<0.001$).

only five treatments using the jar lid substitute host were run with the pufferfish, and thus limited the statistical analysis. Clearly, the trend with the substitute host is similar to the survival times of the control but less than the survival time with the echinoderm (Fig. 3). Ten treatments were run with the pufferfish and *Clypeasterophilus rugatus*. Fig. 4 shows that the survival time of *C. rugatus* with the jar lid was significantly higher than the control ($p=0.008$) but was significantly less than the survival time with the echinoderm ($p<0.001$).

Behavior of crabs and fish in predation trials

Dissodactylus crinitichelis was found on the oral surface of *Encope michelini* when present except for two out of ten crabs. These two were eaten from the aboral surface of the echinoderm by the pufferfish. The crabs spent little time on the substitute host. Instead they attempted to bury themselves in sand on the aquarium bottom. Similar behavior was observed during the control. The

crabs continued to burrow when the fish swam nearby and were quickly eaten after the fish seemed to notice them.

Clypeasterophilus rugatus was also found on the oral surface of its host echinoderm, *Clypeaster rosaceus*, when present. In trials without the echinoderm, *C. rugatus* moved slowly around the aquarium and spent most of its time either along the sides of the aquarium or along the sides of the substitute host. Also in these trials, *Clypeasterophilus rugatus* appeared to stop moving completely when the fish approached. It did not attempt to burrow as did *Dissodactylus crinitichelis*.

4. Discussion

Host preferences

Preference tests are common experimental tools used in behavioral ecology to determine specificity of organisms for many variables, such as food or habitat (Gray et al., 1968; Stevens, 1990; Gwaltney and Brooks, 1994). In the case of symbioses, the symbiont typically must find and select its host. One must be careful, however, when making conclusions about preference without rigorous testing (Gwaltney and Brooks, 1994). Thus, conditioning trials with alternate hosts were done in the present study to determine whether initial preferences were conclusive or the result of transient, recent experiences with one of several "preferred" hosts.

The pinnotherid crab *Clypeasterophilus rugatus*, though observed free-living on rare occasions (Rathburn, 1918; Voss and Voss, 1955), has been found almost exclusively with the sea biscuit *Clypeaster rosaceus* (Telford, 1982; Pohle, 1984; Campos and Griffith, 1990), as was observed in this study. *Clypeasterophilus rugatus* has adaptations (e.g., claws, diet, and coloration) that make it appear more specialized to life on its field host than other pinnotherid crabs to their hosts (Telford, 1982). The genus in which this crab now belongs, *Clypeasterophilus* or "Clypeaster-loving," again reflects the apparent specialization of this crab. These observations allow for the prediction that *Clypeasterophilus rugatus* should exhibit a strong preference for the sea biscuit *Clypeaster rosaceus*. In fact, *C. rugatus* showed a highly significant preference for this host initially (88%). After four weeks of conditioning with the alternate host, *Encope michelini*, the crabs' choice of its normal field host, the sea biscuit, was still significant (79%). Therefore, *Clypeasterophilus rugatus* possesses a real, persistent preference for its normal field host *Clypeaster rosaceus*. Again, the morphological and behavioral specialization of this crab is likely a major factor in determining the species specificity for its host.

Significant differences were found in the behavior of the pinnotherid crab *Dissodactylus crinitichelis* compared to *C. rugatus*. *D. crinitichelis* is widely distributed and has been found on five different echinoderm hosts (Werdning and Sanchez, 1989). *D. crinitichelis* was found on a sand dollar host, *Encope michelini* (one of the five known hosts), and an attempt was made to condition the crabs to the sympatric sea biscuit *C. rosaceus*, though it was not one of the five normal hosts. The apparent generalist strategy for hosts by *D. crinitichelis* allowed for the prediction that its initial host preference could be altered by conditioning. Initially, 74% of *Dissodactylus crinitichelis* preferred the field host, *Encope michelini*. However, after conditioning 79% of *D. crinitichelis* preferred their conditioning host, *Clypeaster rosaceus*. These results show clearly the different strategy employed by *D. crinitichelis* for host selection compared to the species-specific behavior of *C. rugatus*.

Interestingly, crab attrition that occurred during the conditioning period provided additional insight into the behavior of the two closely related, pinnotherid crabs. 86 out of 167 *D. crinitichelis* died during the conditioning period, as they were apparently consumed by the alternate host, *Clypeaster rosaceus* (sea biscuit). Only 4 out of 85 *C. rugatus* died while conditioning with their alternate host *Encope michelini* (sand dollar). *D. crinitichelis* switched its preference to the alternate host despite the threat the echinoderm posed to its survival. Furthermore, *C. rugatus* must possess fundamental differences from *D. crinitichelis* to survive on the potentially crab-killing sea biscuit host.

Previous studies on a third closely related, pinnotherid crab species, *Dissodactylus mellitae*, are relevant for comparison to the present study. Gray et al. (1968) used chemical detection testing to determine host specificity of *D. mellitae*, which has been found on three different hosts. They collected *D. mellitae* crabs from the five-lunuled sand dollar, *Mellita quinquesperforata*, in North Carolina and found that crabs switched host preference (i.e., chemically mediated behavior towards host factors) readily after acclimation to an alternate host (Gray et al., 1968). Although we will be discussing odor responses in the next section, these results are germane here as *D. mellitae* appears to have an ecological role more similar to that of *D. crinitichelis* in terms of host specificity. All three crabs are closely related, sympatric, and have a larval stage dependent on locating the host (Pohle and Telford, 1981; Pohle, 1984), yet differences in host specificity exist. Each crab species appears to have developed an optimal strategy for their specific niche and maintenance of the symbiosis. In the case of the two *Dissodactylus* crabs, behavioral plasticity may provide them with increased opportunities for alternate hosts should populations of one echinoderm host decline or become less suitable. Alternatively, species-specific symbionts are potentially at greater risk should their normal host be unavailable. The present study and comparable studies (e.g., Gwaltney and Brooks, 1994) indicate the need to have a thorough

understanding of the functional and ecological role in terms of costs/benefits to both partners in symbiotic associations.

Chemical detection

The crabs in this study are small with greatly reduced eyes suggesting they may rely heavily on chemical detection in locating their hosts (Telford, 1982). As mentioned in the previous section, Gray et al. (1968) found that *Dissodactylus mellitae* responded to chemicals from both their original host and a new host after acclimation (conditioning). In this study, no significant use of chemical detection was found for either *Clypeasterophilus rugatus* or *Dissodactylus crinitichelis*. In contrast, studies involving other pinnotherid species (Derby and Atema, 1980; Brooks and Rittschof, 1995) used a similar apparatus to Fig. 1 and similar protocol and found a positive chemical response. Gray's study found a positive chemical response by *D. mellitae* using a trough apparatus (Fig. 2). Though *D. mellitae* shares the characteristic of having larval stages dependent at one point on contact with the host (Pohle and Telford, 1981; Pohle, 1984), chemical detection may be more important during the larval stages for *C. rugatus* and *D. crinitichelis*. The results found in this study illustrate that differences in the behavior of adult pinnotherids living with echinoderms exist. Future studies should focus on the chemical detection of different larval stages to determine if chemically mediated responses diminish over the lifetime of the crab.

Protection

Organisms involved in symbioses, by definition, must associate (if not always, at least part of the time). There is a presumed benefit to one or both partners; otherwise, why live together. Previous studies have indicated that food source may be one possible benefit to the crab (Telford, 1982; Bell, 1987). This study explored the additional possibility that crabs may receive protection by living with echinoderms. Clearly crabs with echinoderms gain some degree of protection from predatory fish. Both *C. rugatus* and *D. crinitichelis* survived significantly longer with the echinoderm than without. When with the substitute host (jar lid), *C. rugatus* received a level of protection intermediate between the echinoderm and no protection, suggesting the distinctive characteristics of the echinoderm provide the most effective protection. Trials involving *D. crinitichelis* and the substitute host were not statistically different; however, a larger sample size would likely provide results similar to those of *C. rugatus*.

Protection from fish was not absolute. Two *D. crinitichelis* on the aboral surface of the sand dollar, *E. michelini*, were consumed while no other crab of

either species was consumed when with an echinoderm. Other crabs were on the oral (bottom) surface of the echinoderm where there may be an advantage of enhanced protection. However, the disadvantage of being on the aboral surface may be reduced in the field as the echinoderms may camouflage themselves with debris or burrow, making the crab difficult to detect by predators. Though it appears that both echinoderms offered protection to the crabs, the protective value of each echinoderm species may be enhanced by the adaptations of the crabs to life on the respective echinoderm hosts. For example, *C. rugatus* has slender claws for excising tissue from between the sea biscuit's spines, and is colored to blend with the sea biscuit thereby appearing as debris atop *Clypeaster rosaceus*. Also, *D. crinitichelis* blends well with sand and has spine-gripping/clipping claws probably advantageous for living on a burrowing echinoderm. Telford (1978) found that typical *D. crinitichelis* aggregation is either two adults (male and female) or several juveniles per host. He also stated that they move to another host if crowding occurs and if the hosts are too far apart, they stay on their current host.

The advantage of protection is one that is used by several crustaceans (e.g., hermit crabs and shrimp) in symbioses with a variety of hosts (see Brooks and Mariscal, 1985; Brooks and Gwaltney, 1993; Mihalik and Brooks, 1997). The present study is the first to demonstrate protection for pinnotherid crabs associating with echinoderms, and provides a fundamental clue as to why these crabs are symbionts.

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