THE RELATIONSHIP BETWEEN HOLOTHURIANS AND THE PORTUNID CRAB *LISSOCARCINUS ORBICULARIS*

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Symbiosis, the close relationship of organisms of different species, can often occur in its various forms of mutualism, commensalism, or parasitism in the crowded marine environment. Sea cucumbers (Family Holithuriidae) are known to host not just one, but a number of symbionts. This study examines two of the symbionts common to holothuroideans, parasitic eulimid snails and the commensal crab *Lissocarcinus orbicularis*, both of which are found on the sea cucumbers *Holothuria atra, Holothuria whitmaei*, and *Actinopyga obesa*. The objectives of this study were threefold: first, to establish whether a relationship exists between the abundance of *L. orbicularis* and the abundance of eulimid snails; second, to determine whether *L. orbicularis* would alter carapace color or patterns in response to environmental changes; and third, to see if crabs would preferentially settle back on their original host cucumbers given an option of a new host. Analyses conducted from study sites on the eastern side of the island of Hawaii show high infestation rates of the snails on *H. atra* and *H. whitmaei*, with low infestation rates on *A. obesa*. Crab occurrences were common on *H. atra*, occasional on *A. obesa*, and rare on *H. whitmaei*, with no significant difference in snail abundances between cucumbers hosting crabs and cucumbers without crabs. Observations in the laboratory revealed changes in color and carapace pattern during crab molting. Crabs did not exhibit fidelity to their original hosts when given the option. These findings may help provide a better understanding of invertebrate symbiotic associations and how, within these associations, fulfill their respective ecological roles.

Introduction

In the aquatic world, many forms of symbiosis take place among a wide variety of organisms. A form of symbiosis where both organisms benefit is mutualism, such as zooxanthellae providing nutrients for coral while having access to the metabolic wastes of the coral. Another form of symbiosis is commensalism, where the symbiont benefits and the host is neither harmed nor helped; for example, a remora attaching itself to a shark and feeding off scraps dropped by the predator. Parasitism, where the symbiont is dependent upon the host to the host's detriment, is illustrated by an isopod living on the tissues of a fish.

Sea cucumbers, class Holothuroidea, are host to many symbionts: turbellarians, polychaetes, gastropods, bivalves, copepods, crabs and fish (Barel 1977). These symbiotic relationships can be commensal, parasitic, or may be undefined, and can take place either externally (ectosymbiotically) or internally (endosymbiotically) on or within the cucumber. Two groups of ectosymbionts of holothurians are crabs, order Decapoda, and snails, class Gastropoda. Various studies have been conducted on crabs and their echinoderm hosts, which can include sand dollars (George and Boone 2003), sea urchins (Thiel et al. 2003a), and sea cucumbers. Crabs on sea cucumbers can either be endosymbionts, such as the pea crab from the family Pinnotheridae which lives in the gut, cloaca, or respiratory trees of sea cucumbers (Hamel et al. 1999; Ng 1999), or ectosymbionts, such as the portunid crab *Lissocarcinus orbicularis*, which lives externally on the sea cucumber, usually near the mouth or anus (Cheng 1970; Hoover 1998; Lyskin and Britayev 2003). The association between *L. orbicularis* and its cucumber host has been described as commensalistic (Crosnier 1962), unlike the relationship between the cucumber host and various parasitic gastropods, which feed on the cucumber's body fluids (Boss 1982; Kato 1998; Bouched and Luetzen 1980; Caso 1968; Rybakov and Yakovlev 1993).

Though many studies have been done on individual symbionts on echinoderms, I have found no published material on holothurians in Hawaiian waters and the relationship between them and their ectosymbionts, the commensal crab *L. orbicularis* and parasitic molluscs from the family Eulimidae. The goal of this study will be to examine these associations. In Hawaii, *Holothuria atra* and *Actinopyga mauritiana* have been associated with the eulimids *Balcis aciculata, Balcis inflexa, Balcis solidula, Eulima metcalfei*, and *Mucronalia nitidula* (Kay 1979). *L. orbicularis* is most commonly associated with *H. atra* (Crosnier 1962; Kay 1979; Lyskin and Britayev 2001b). From recent observations, I have also found eulimids, as well as *L. orbicularis,* on *Holothuria whitmaei* and *Actinopyga obesa.* The holothurians included in this study are *H. atra, H. whitmaei, A. mauritiana*, and *A. obesa.* Although the literature has not shown *A. obesa* to host either the crab or the parasitic snail, it was included to see if there were occurrences of either symbionts.

The focus of this project was to better understand the symbiotic relationship between holothurians, parasitic snails, and their commensal crab *Lissocarcinus obicularis*. The questions addressed are: What is the relative frequency of occurrence of *L. orbicularis* on the various species of sea cucumbers? Does the relative abundance of eulimid snails have any relationship to the presence or abundance of *L. orbicularis*? Do crabs exhibit fidelity for sea cucumber hosts?

Methods

Field collections and observation

Between September 24, 2004 and February 19, 2005, a total of 167 sea cucumbers were collected from three locations on the eastern side of the island of Hawaii — Richardson's Beach Park, Onekahakaha Beach Park, and Kapoho Tide Pools — for subsequent examination for symbionts. Collections took place approximately every week, weather and conditions permitting. Of the total sea cucumbers sampled, 137 came from Richardson's Beach Park, 19 were collected from Kapoho, and 11 came from Onekahakaha. To help prevent resampling of a previously sampled cucumber, each site sampled was broken down into subsites separated by at least a 10 m buffer zone. Three species of the cucumbers (*H. atra, H. whitmaei,* and *A. obesa*) were collected along with their symbionts, the commensal crab *L. orbicularis* and the parasitic snails from the family Eulimidae.

The collections were made while snorkeling at depths of approximately one to two meters. The cucumbers were placed in numbered plastic bags and brought to shore, where the sand and any other debris was brushed off the cucumber and collected in the plastic bag, and a visual inspection for *L. orbicularis* was made. When *L. orbicularis* was present, it was removed and the carapace was measured using calipers, and its color and approximate location on the cucumber was noted. The cucumber was then removed from the plastic bag and placed in a bin filled with seawater to acclimate, after which its length and width were measured and noted, along with the date, time, species of cucumber, location, and depth of collection. The acclimation process also allowed for closer inspection for crab symbionts. Many symbionts take refuge in their hosts when they are disturbed; *L. orbicularis* is often found in or near the oral tentacles of the sea cucumber (Lyskin and Britayev 2001b; Cheng 1970). The cucumbers normally took 5 to 30 minutes to relax and extend their oral tentacles, whereupon any crabs hidden within the tentacles could be seen and carefully removed. After all measurements were taken, the cucumbers and crabs were returned to the ocean.

Laboratory observations and experiments

In the laboratory, each sand sample was examined using a dissecting microscope, and eulimid snails were removed and counted. Representative samples of the snails were identified and measured. Statistical analysis was performed using Minitab to determine whether or not the presence of *L. orbicularis* had an affect on the snail burden on the cucumber.

Collections were also made to gather cucumbers and their symbionts for observations in the lab. A number of crabs were removed from their host sea cucumbers and kept in small, aerated aquaria, either singly or with up to three other crabs, to observe growth and behavior. Crabs were fed commercially bought fish flakes on a weekly basis. Several sea cucumbers along with their crab symbionts were kept in larger, aerated and filtered aquaria for general observations. These larger aquaria also housed a number of other invertebrates, including sea urchins, brittle stars, various shrimp, cowries, and sea anemones.

Tests were run to observe whether crabs would exhibit fidelity to their sea cucumber hosts when given a choice of hosts. Six trials were run, with three replicates each, with new cucumbers being used for each trial. Five of the trials used *H. atra* – *H. atra* pairs; one trial used a *H. whitmaei* – *H. whitmaei* pair. For each replicate, a crab would be removed from its host sea cucumber. A plastic bin was prepared by filling it with seawater and providing aeration with an air stone. The host sea cumber would be placed at one end of the bin, and a cucumber of the same species was placed at the other end of the bin. The crab was then placed at a location equidistant from both cucumbers, and was observed to see which cucumber it would attach to. This information was recorded, along with the time elapsed from placing the crab in the bin to attachment to the cucumber. The cucumbers and crabs were allowed to acclimate for one hour between replicates.

Results

Crab and snail abundances

A total of 748 Eulimid snails and 70 crabs of the species *L. orbicularis* were found on 167 sea cucumbers. Of the sea cucumbers collected, most were *H. atra* (51%), followed by *H. whitmaei* (35%), then *A. obesa* (14%) (Fig. 1). Cucumbers ranged in length from 9 to 39 cm, with an average length of 26.4 ± 0.38 cm (mean ± SE). All cucumber species were examined for presence of *L. orbicularis*; 34% of the cucumbers examined had crabs. The cucumber species with the highest occurrences of *L. orbicularis* was *H. atra* (56%), followed by *A.* obesa (17%), and *H. whitmaei* (5%) (Fig.2). Of the 56 individual sea cucumbers that hosted crabs, 20% hosted male/female pairs. Of the total number of crabs, 56% were female, 33% were male; I was not able to identify gender of the remaining 11%. Female crabs tended to be larger, with carapace lengths averaging 8.7 ± 1.9 mm, compared to male crabs, which had average lengths of 6.2 ± 2.4 mm. In 66% of instances where crabs were on sea cucumbers, the crabs were found in the cucumber's oral tentacles. In all other cases, the crabs were found on the exterior of the cucumber.

Sea cucumbers were also examined for the presence of parasitic eulimid snails, with 78% of cucumbers showing infestations. Of the total number of cucumbers with snails, *H. atra* was the most frequently infested (89%), followed by *H. whitmaei* (86%), and *A. obesa* (18%) (Fig. 3).

Of the 159 sea cucumbers examined for both crab and snail symbionts, no significant difference was found between the median number of snails on cucumbers with crabs versus the median number of snails on sea cucumbers without crabs (Mann-Whitney, $P = 0.6721$, $W = 8834.0$) (Fig. 4), however, power analysis indicates that the sample sizes may have been insufficient (Power = 0.146336, differences = .794, standard deviation = 4.376). Sea cucumbers that did not host any crabs had an average snail infestation of 5.0 ± .0479 (mean ± SE); cucumbers that hosted a single crab had an average of 4.0 ± 0.649 snails; cucumbers with two crabs had an average of 6.0 ± 1.90 snails, and in the two instances that cucumber hosted three crabs, no snails were found.

Crab carapace color changes

A number of *L. orbicularis* specimens were kept in aerated aquaria in the laboratory for observation and behavioral testing. Though not all survived, a number have lived for more than a month without a sea cucumber; one crab lived in aquaria for almost five months. One juvenile crab, which was originally removed from *H. atra* and was housed in an aquarium with light-colored sand, exhibited a change in carapace coloration and patterning as it molted over a two-month period. As time progressed, the carapace coloration on this crab changed from predominantly black with white spots to predominantly white with black spots (Fig. 5).

Of the crabs found on the sea cucumbers, most (93%) had dark brownish-red or black carapaces with white spots, but a small percentage (7%) of the crabs found had white carapaces with brown or olive-colored spots. Four of these lighter crabs were found associated with *H. atra*, and one was found on *A. obesa*; none were found on *H. whitmaei* (Fig. 6). Two of the lighter crabs were kept in aquaria with darker sand bottoms. After two months, both crabs appeared to have molted, as measurements showed an increase in size in both cases, but only one crab has shown a change in color, with a darkening and increase in number of spots (Fig. 7).

Behavioral testing for crab host preference

When removed from their original cucumber hosts, crabs took between one and six minutes to attach to a sea cucumber, and did not show a significant preference for reattachment when given a choice between their original host cucumber and a new host of the same species ($\chi^2 = 1.26$; DF =1; P>0.25).

Eulimid species found

The most common eulimid snail species found on all species of cucumbers appeared to be *B. aciculata* and *M. nitidula*, which infested both *H. atra* and *H. whitmaei*, while *A. obesa* appeared to be only infested with *M. nitidula.*

Additional observations

A number of incidental invertebrates were noted on the sea cucumbers as well as in the sand samples. These organisms were not quantified nor identified to genus or species, but included a variety of sea hares, amphipods, isopods, polychaete worms, brachyuran crabs, and gastropods; the exact nature of their associations is unknown. Six crab megalopae were also found, but specific identifications as to species were not possible, although descriptions of *L. orbicularis* have been published (Lyskin, 2001). However, preliminary identification indicates that two of the megalopa could be *L. orbicularis* (Lyskin, personal communication).

Discussion

Crab and snail abundances

Of the species of cucumbers collected, *H. atra* was the most abundant, and had the highest occurrences of total symbionts. *L. orbicularis* occurred in 56% of *H. atra* in this study, compared to 75% on *H. atra* in Oahu, Hawaii (Mauerman 2002), and up to 85% of various holothurian species hosting crabs in Vietnam (Lyskin and Britayev 2001). It may be that *H. atra* is a more suitable host for crabs than *H. whitmaei* and *A. obesa*. Although *H. whitmaei* has dark coloring and sand coating similar to *H. atra*, its body is harder, and perhaps is more difficult for the crabs to maintain purchase on. The more pronounced papillae on the dorsal surface of *H. atra* may makes its skin easier to grasp. During removal of the crabs from the sea cucumbers, the crabs were often able to grasp tightly to the skin of *H. atra*, making removal difficult. *A. obesa* may have been the least hospitable, with its golden-brown color providing inadequate camouflage for darker crabs, and little to no sand coverage providing insufficient camouflage for lighter crabs.

In Oahu and Kauai, 60% of *H. atra* examined were infested with eulimids (Kay 1979). The percentage of eulimids found on *H. atra* in this study was somewhat higher, with 89% being infested. The more pliable skin of *H. atra* may also explain the greater number of eulimids present on this species, as the snails would more easily be able to insert their proboscis into the cucumber's body wall (Boss 1982; Warén 1983), and as with the crabs, the coating of sand may also help provide cover for the snails.

There was no significant difference between the presence of crabs and the abundance of eulimid snails. And although insufficient sample sizes prevent confidence in this conclusion, there are some possible explanations for this lack of interaction. The crabs feed mainly on bottom-dwelling organisms (Lyskin and Britayev 2003) and were found predominantly in the oral tentacles of the cucumbers, while the snails feed mainly on cucumbers' tissue and fluids (Boss 1982; Warén 1983) and were located on the tegument of the cucumbers. This compartmentalization of feeding and habitat niches may explain the lack of competition, and therefore lack of connection, between the two symbionts. Also, an aid in deterring predators may be the morphology of the snail's shell; its smooth, hard, slick, surface makes it difficult to grasp (Warén 1983). Alteration of shell morphology has been illustrated by the blue mussel *Mytilus edulis,* which is able to clean itself of attached barnacles as a defense against predation. Predatory crabs have shown a preference for mussels that are barnacle-fouled; the barnacles likely allowing the crabs to more easily grip and subsequently crush the mussels (Enderlein 2003).

Crab carapace color changes

One hypothesis for the change from dark to light carapace color in the first crab is that it was attempting to change its pattern to better blend in with the background of light-colored sand. The crabs are normally well camouflaged on the sand-covered surfaces of the black sea cucumbers. The second crab, with the white carapace that exhibited a darkening and increase of spots when placed on darker sand may also have been attempting to better blend in with the background. The second crab did not have a change as dramatic as the first crab, which may be because the second crab was larger than the first crab, which molted more frequently.

The idea of marine organisms using protective coloration in their various associations to take refuge dates back to the late 1800's, with the discovery of small blue crabs clinging to a blue-shelled mollusks, and white-spotted yellow shrimps and crabs hiding in the yellow Gulf-weeds with patches of white bryozoans (Wallace 1889). More recent discoveries of homochromy — coloration that matches that of the host — include the reddish-green porcellanid crab *Allopetrolisthes spinifrons* occurring on the red-green sea anemone *Phymactis clematis*, which may offer the crab protection (Thiel et al. 2003b). Nudibranchs have also used cryptic coloration and morphology to avoid predation while taking refuge on soft corals (Avila 1999).

Behavioral testing for crab host preference

Laboratory observations demonstrated that crabs did not show preference for returning to their original host sea cucumber or to a new host when given a choice. This is in agreement with casual observations made in the field. When crabs were removed from their host cucumbers for measurements and sex identification, and then returned to a bin containing two to four sea cucumbers, they appeared to attach to whatever cucumber was closest. Host fidelity of symbionts has been shown to be affected by whether the costs of moving to a new host are balanced by the benefits of doing so (Thiel et al. 2003b).

Conclusion

Observation and experimentation yielded interesting results that may shed light on the symbiotic associations between sea cucumbers and their symbiotic snails and crabs. There was no significant difference in snail abundances between sea cucumbers hosting crabs and cucumbers without crabs, demonstrating a possible lack of competition due to compartmentalization of feeding and habitat niches. Changes in crab color and carapace pattern during molting may suggest use of homochromy, or protection coloration to blend in with the host background. Crabs did not exhibit fidelity to their original hosts when given the option, possibly indicating that proximity, rather than host specificity, dictates crab host preference.

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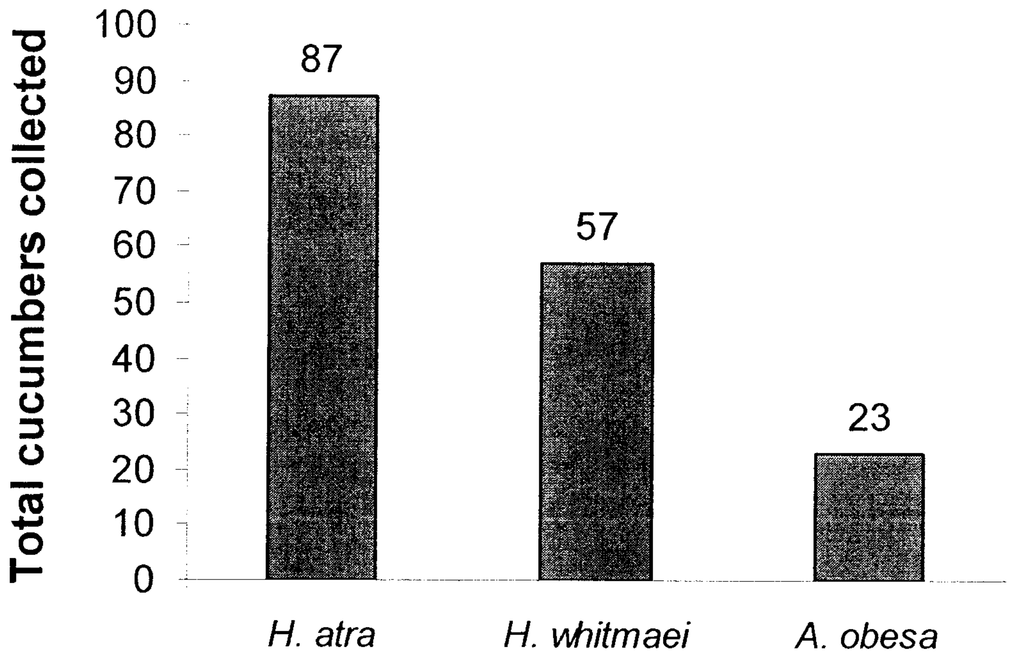

Figure 1. Total number of cucumbers collected, by cucumber species.

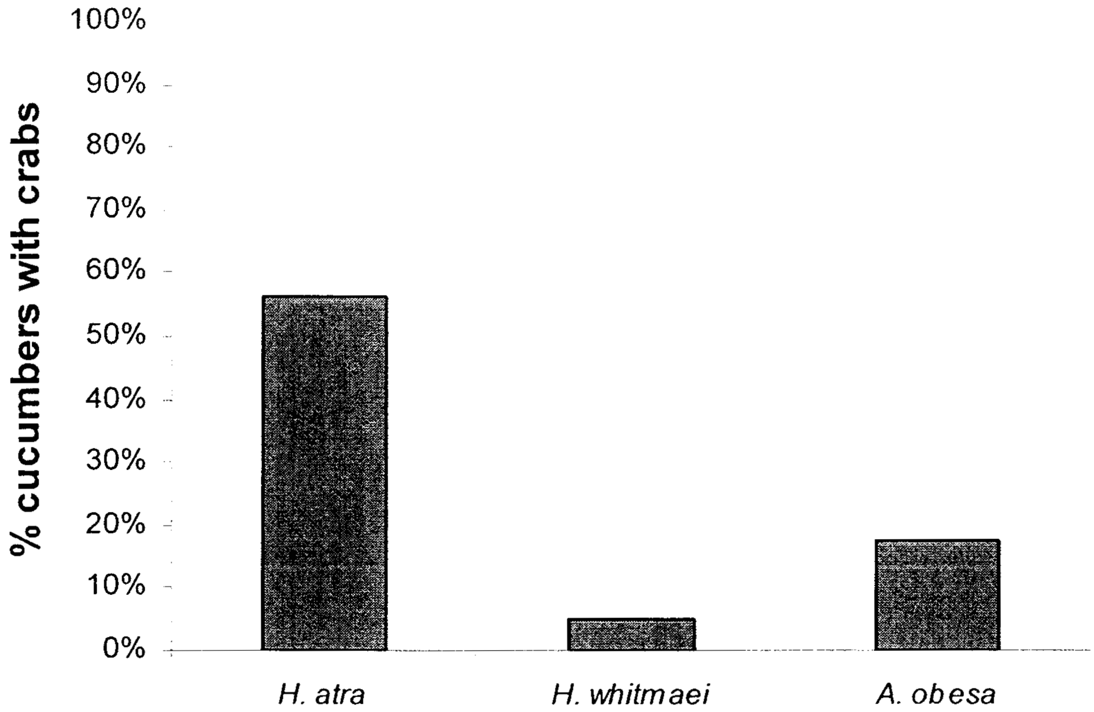

Figure 2. Percent of cumbers hosting crabs, by cucumber species.

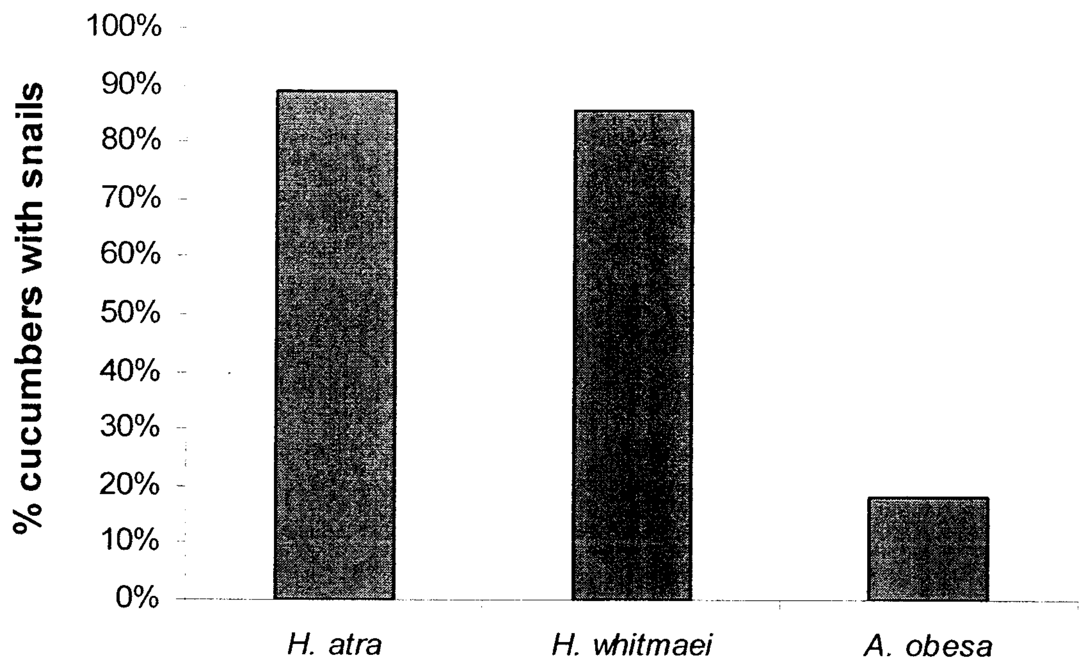

Figure 3. Percent of cucumbers hosting snails, by cucumber species.

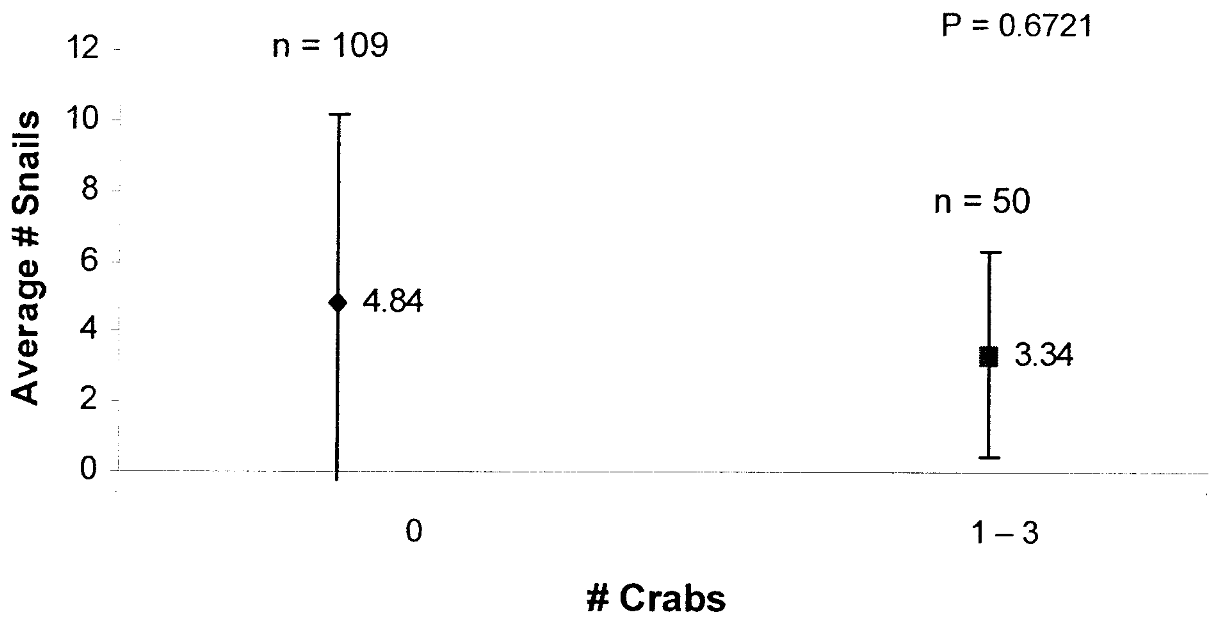

Figure 4. Relationship between presence of crabs and abundance of snails found on sea cucumbers. n = number of cucumbers collected.

Figure 5. Photos of *L. orbicularis* taken over a two-month period. (Image scale not uniform; not intended to indicate growth.)

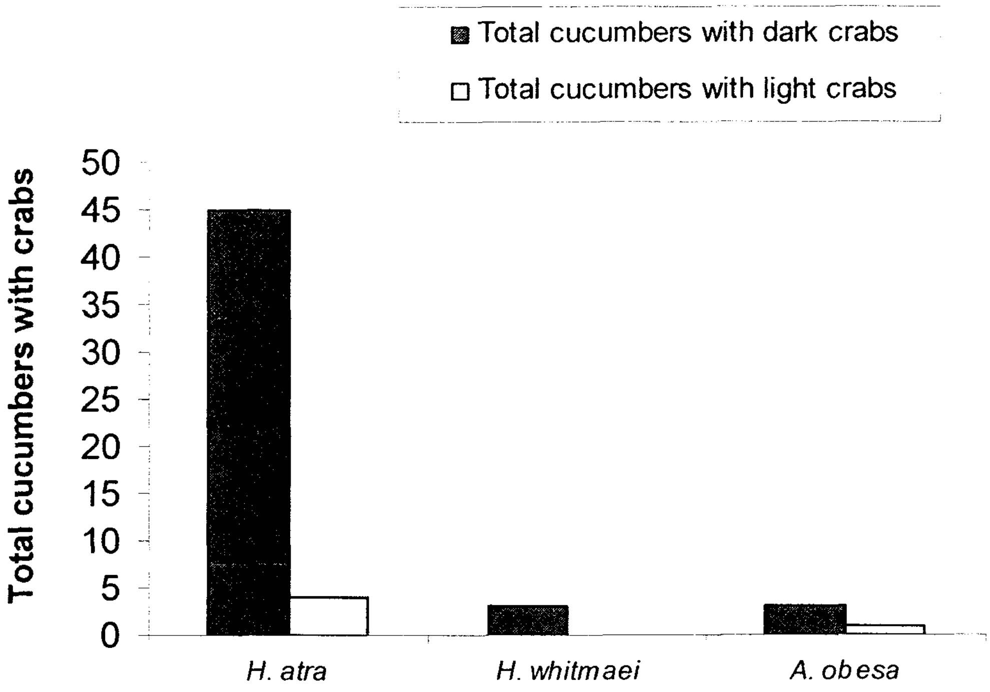

Figure 6. Cucumbers hosting crabs with dark carapaces with white-spots (gray columns) or light carapaces with dark spots (white columns).

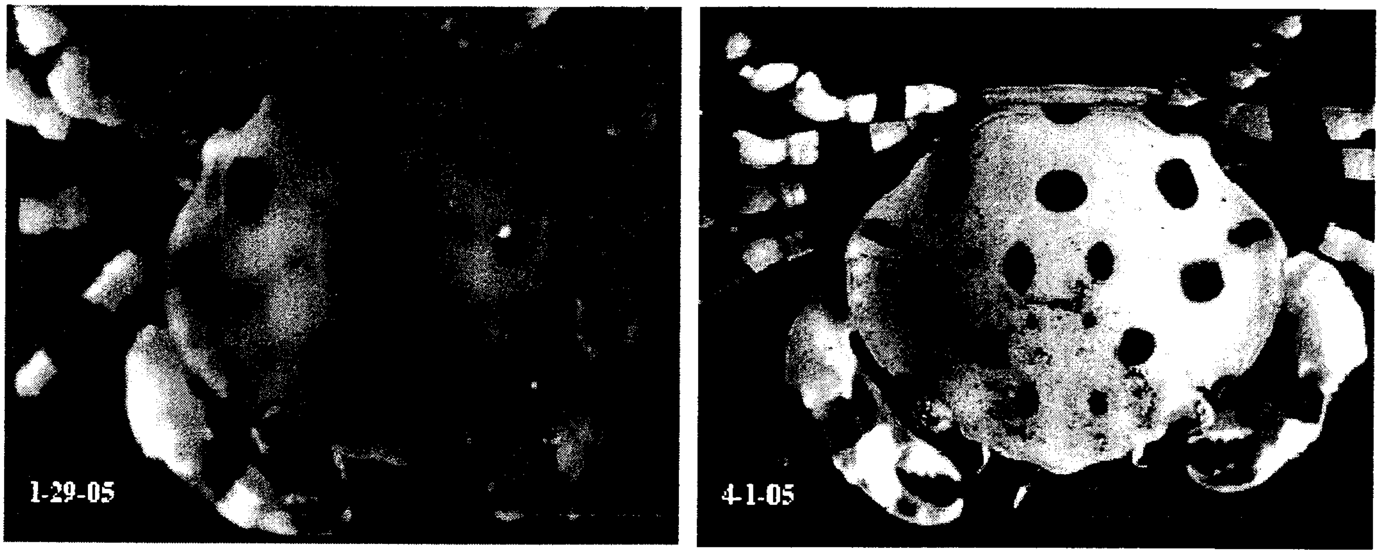

Figure 7. Photos of *L. orbicularis* taken over a two-month period. (Image scale not uniform; not intended to indicate growth.)