The symbiotic relationship between Sea cucumbers (Holothuriidae) and Pearlfish (Carapidae)

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Abstract: The relationship between sea cucumbers (Holothuriidae) and pearlfishes (Carapidae) is a relatively unknown system. In this study, we set out to answer questions regarding the nature of this relationship; i.e. parasitic, mutualistic or commensal. The Holothuridae species we studied were *Thelenota ananas* and *Bohadschia argus* and the Carapidae species we examined were *Encheliophis Boraborensis* and *Carapus homei.* We measured the distribution of holothurians by performing transects at three sites in Moorea, French Polynesia. We collected Holothurian hosts with potential Carapus occupants over a four-week time period. We ran a series of experiments at C.R.I.O.B.E. research station. We observed that host specificity was not significant in this system. However, interspecific and intraspecific competition between the carapidae species was present. Species association of Carapidae with location of Holothuriidae was also prominent.

Introduction

Holothurians are members of the phylum Echinodermata. These organisms occur throughout the world's oceans and are usually found in shallow reef areas and in areas of sand and rubble. Most species feed on the organic film that coats the sandy bottom by passing sand through their digestive tract and excreting the unused matter (Allen and Steene,1994). Some species release sticky threads called Cuverian tubules to ward off predators (Gosliner, Behrens and Williams, 1996). In more serious predatory situations, the cucumber may eviscerate its internal organs as an offering to prevent more important tissues from being eaten, and will then later regenerate those organs.

The family Holothuriidae serves as a host to several species of pearlfish. The family Carapidae are commonly found living in the body cavities of several invertebrates including asteroids, bivalves and holothurians. These fish are usually found in shallow to moderately deep water. Although not much is known about their larval metamorphosis, they are thought to have two distinct larval stages. The planktonic larval stage is dubbed the vexillifer stage and occurs directly after the hatching of the egg. The larvae then metamorphose into the tenuis stage in which the body lengthens and the animal becomes

benthic, some finding hosts to live in such as cucumbers. The juvenile stage develops after settlement inside the body cavity of a sea cucumber and a final thickening of the body occurs in which the carapid is then considered an adult. During the tenuis stage, the larvae are thought to be parasitic and then gradually assume a commensal relationship with its host (E. Parmentier, A. Lo-Yat, and P. Vandewalle 2002).

The objective of our study was to determine the nature of the interaction between the pearlfish and their host and to and to look at aspects of a probable commensal relationship. Specifically we addressed the following questions: (1) Does competition occur between *E. boraborensis* and *C. homei* for a host? (2) Are carapids host specific and do they return to their original host? (3) Are Carapidae and Holothuridae association cues present? (4) Does carapidae nocturnal activity occur?

We found through surveys and experiments that host specificity was not significant for the Carapidae. Our experiments suggest that interspecific and intraspecific competition does occur between Carapidae species. Using statistical analysis, it was found that there was a strong correlation between Carapidae species association and Holothurian host location.

Specific Hypotheses

H1 Competition occurs between *E. boraborensis* and *C. homei* for different species of cucumber hosts and occurs when more than one species is placed with one host.

H2 When presented with different species of cucumbers, pearlfish will prefer its present host to other potential Holothuriidae host.

H3 Association cues such as carapids “knocking” on the cucumbers anus are given by potential occupants to encourage entry and exit from their hosts’ body cavity.

H4 Pearlfish exit their host at night for feeding.

Methods

1. Study site. We chose sites just outside Opunohu Bay on the island of Moorea in French Polynesia located at 17°30’S, 149°50’W. White House Wall, Public Beach,

and West Opunohu were the sites selected to collect and survey the abundance and distribution of the two species of host sea cucumbers. White House Wall (WHW-149° 51 097') is a sloping reef wall that ranges from 10 feet in depth to 50 feet in depth where it levels off to a sandy floor. Public Beach (PB-149° 50 973') is a fairly shallow site with depths ranging from 5 feet to 15 feet. The topography consists of a fringing reef that leads into a patchy crest reef. West Opunohu (WO-149° 52 253') is located on the edge of the channel just outside of Opunohu Bay. This site is also fairly shallow in depth ranging from 3 feet to 10 feet. West Opunohu contains a sandy floor with a patchy crest reef.

2. Study species. Two species of Holothurians were used for this experiment. *Thelenota ananas* is a large species reaching up to a meter in length. The body is black with orange conical papillae arranged in a stellate fashion. *T. ananas* is found 5 to 30 meters in depth and inhabits the interface between reefs and sand (Behrens, Gosliner and Williams 1996). *Bohadschia argus* is a common species and is characterized by two color morphologies. The colors range from a light gray to a dark reddish brown both containing a mottled spotting pattern. *B. argus* inhabits shallow water reef and rubble habitats and range in waters from 5 to 20 meters in depth (Behrens, Gosliner and Williams 1996).

Carapidae are eel-like fishes that lack scales and pelvic fins but have pectoral fins. Although most species of Carapidae live in the body cavities of invertebrates, some are found to be free living up to 600 meters in depth (Myers 1999). Two species of carapids were used in this study. *Encheliophis boraborensis* is brownish in color and has very small eyes. They possess small, stellate melanophores that are visible near the jaws and increase in number with age (Markle and Olney 1990). *E. boraborensis* are the only species of carapidae that have melanophores present on the anal fin (Parmentier, Lo-Yat, Vendewalle 2000). They have an extremely elongated body and an enlarged swim bladder, which takes up ninety percent of it visceral cavity. *C. homei* is transparent in color and silvery patches are apparent in the abdominal section of the body. The eyes are extremely enlarged and melanophores are present along the body, however, they are

somewhat hidden (Markle and Olney 1990). They also have an elongated body as well as an enlarged swim bladder.

3. Surveys. Using scuba and free diving techniques, we performed collections at the three sites outside Opunohu Bay. At each of the sites, we did four 50 meter transects, two of which were shallow and two of which were deep. Cucumbers were counted on 5 meters of each side along the 50 meters. We then used the transects to measure the distribution and abundance of the Holothurian hosts.

4. Experiments. Collections of the two species of Holothurians occurred from Nov 8th to Dec 2nd of 2002. Specimens were collected at various depths and time periods throughout the day to examine Carapidae distribution patterns and to be used in our experiments. The collected specimens were brought back to the lab, placed in salt-water tanks, and observed daily and nightly. Pearl fish were extracted by depleting the hosts of oxygen using a shallow container of water and confining the cucumber in the container until the carapid exited its host.

To test the idea that there was intraspecific and interspecific competition (H1), we tagged carapidae and its hosts in the lab to observe if competition occurred between the tagged carapidae and different carapidae for hosts. Competition was measured by observing aggressive behavior (biting, cannibalism) between the same species as well as different species of carapidae.

In order to assess the concept of host specificity (H2), we tagged pearlfish and placed them in a tank with two or more species of sea cucumber including its present host. Observations were made of the twelve trials on the behavior and decisions of the tagged pearl fish as to what host it decided to enter.

The presence of association cues between the Holothurians and Carapids (H3) was investigated by the following method. The tagged pearl fish was introduced to a holothuriidae host and observations of association cues (i.e. tapping near anal duct, smelling, listening) were made in a lab.

To test the idea that carapids were nocturnally active (H4), night observations were done to see if the fish really did exit its host for feeding purposes.

Finally, to see if we could find evidence for parasitism (Trott reported evidence of parasitism in 1972), we dissected pearlfish and examined its stomach contents for cucumber remains.

Results

Intraspecific and interspecific competition between two carapid species for a Holothurian host

Intraspecific and interspecific competition was found in both species of carapids. *E. boraborensis* demonstrated aggressive behavior toward its own species as well as toward *C. homei*. It was found that competition occurred inside the body cavity of the cucumber between several carapids. In one trial, two male *E. boraborensis* fought until death occurred. Dissection of the host cucumber took place finding one male, one female, and an adult *C. homei*. Within the stomach of the surviving male *E. boraborensis*, a juvenile *C. homei* was found suggesting cannibalism occurs due to competition. This was verified in another replication as a juvenile *C. homei* was once again found in an adult *E. boraborensis*. Other evidence of competition included bite marks on the specimens used in these trials and on other carapids extracted from collected cucumbers. In one such case, evidence of interspecific fighting between the two species of carapids was observed.

Host specificity of Carapidae in relation to Holothurian hosts

After monitoring 12 trials of host preference, the results indicated no selectivity of carapids for their original hosts. Both species of carapids either entered the first host that they came across or hosts other than their own. In 12 trials there were only 2 cases in which the fish did find its original host. We performed these trials with 2 adult *C. homei*, 1 juvenile *C. homei*, 7 *E. boraborensis* adults, and 2 *E. boraborensis* juveniles.

Association cues given by potential carapid occupants to Holothurian hosts for entry and exit into the body cavity

In the 24 trials of host specificity of intraspecific and interspecific competition, association cues between the fish and its potential host was observed. Each fish appeared to smell the length of the body of its potential host, doing lengthy surveys a number of times before actually entering the anus. Both species of carapids demonstrated this behavior. It was also observed that both species of carapids seemed to listen along the body of the host almost as if trying to detect the presence of another occupant inside. A type of knocking around the anus area was initiated by the fish as a means of encouraging its entrance into the cavity.

Nocturnal activity of two species of carapids

After one all night survey of a *B. argus* that was occupied by two *E. boraborensis* and two *C. homei* observations revealed no nocturnal activity of the fish from 8 pm until 6 am.

Discussion

Many exciting observations were made throughout this venture and several of the statements made in our references were disproved with our findings. Many reported pearlfish as entering tail first, but we found headfirst entry prominent among our many specimens (Myers 1991). *C. Homei* was reported as living independently from other species, while out of four cases of hosts containing multiple carapids, we found this species living with *E. Boraborensis* three of those instances (Trott and Trott 1972). It was stated in Micronesian Reef Fishes among other places that the pearlfish come out to forage at night, but our observations presented different information (Myers 1990). No foraging occurred during our tests, but these observations were done in aquaria and could be construed do to stress of the specimens.

Evidence of competition was prominent among our observations. Initially, we expected to observe competition interspecifically, but not intraspecifically. However,

during out trials it was discovered that in fact aggression was both interspecific and intraspecific. Though our experiments had a more elevated competitive environment than what was seen in the wild and this provided accurate tests to show that competition was in fact present both interspecifically and intraspecifically. During our trials, tail biting was the most common form of aggression seen, as were few instances of cannibalism.

Before our trials began we expected to find to host fidelity among the carapids and their hosts. However, after twelve trials we found that host preference was in fact not significant and most of the carapids chose to reside in the cucumber that was nearest them. We also expected that a carapid would be able to locate its original host during the trials, but that was not found to be true.

Initially we believed that association cues were present for the entry of the Carapid into its Holothurian host. After numerous observations, it was determined that this was true. The Carapidae was observed to perform several knocking and pecking motions at the cloaca of the Holothurian in order to communicate its entry.

During our assessment of distributions we found a significant difference between location sites of the two carapidae host species with *B. argus* only found in the PB site and *T. ananas* found in WHW and WO in smaller numbers (Figure 1).

E. boraborensis was more often found at WHW and PB than at WO. *C. homei* was more apparent at PB than WHW, and was not even present at WO (Figure 2). There were two cases at PB in which both species were found in one host as well as one instance at WHW. It was also more likely to find no fish in a host from WHW than from a host at PB and WO.

Looking at the pattern of age distributions among fish specimens collected, a two-way ANOVA test revealed a p-value of 0.059, indicating a significant difference between location and age of carapids. A large population of adult carapids was found at WHW and was prevalent to the population of juveniles. At PB and WO, we found an equal amount of both ages (Figure 3).

Color morphology of *B. argus* showed that *E. boraborensis* was more prevalent in the light colored morph than in the dark colored morph (Figure 4). However, *C. homei* was found to be equally present in both.

Although our project provided a great deal of invaluable information, there are still many unanswered questions. For example, it would be interesting to conduct further research on the life history of the Carapidae and more specifically, activity within the Holothurian. During dissections of Holothurian hosts, we found carapidae specimens lodged within the respiratory trees and, in once instance inside the gut. We also observed the Carapid entering the Holothurian headfirst and exiting headfirst. Using such devices such as a sonogram or a hydrophone would be useful in further investigation of the orientation and observation of the Carapid.

Another aspect to research further would be if the Carapidae actually leaves its host to feed, or whether it feeds off the insides of it's host. During the housing of our specimens we observed carapidae eating through its host in order to exit its host. This behavior was observed but has not been addressed in any other literature.

In the literature it suggests that due to specialized morphology, *E. boraborensis* is parasitic to their hosts. This Carapidae species has well developed jaws and fang-like teeth. Their small eyes and behavior indicate that they would not need to leave their hosts. In contrast, the *C. homei* has larger eyes and teeth that would be more appropriate for foraging behavior (E. Parmentier, A. Lo-Yat, and P.Vandewalle 2002). Due to the nature of the behavior observed, this appears to be true.

Although our data remained significant throughout the duration of the project, many problems could have been prevented with a more adequate sea water system. However, we strongly believe that the answers to our questions were strongly supported by our data.

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Figure 1: Host Location

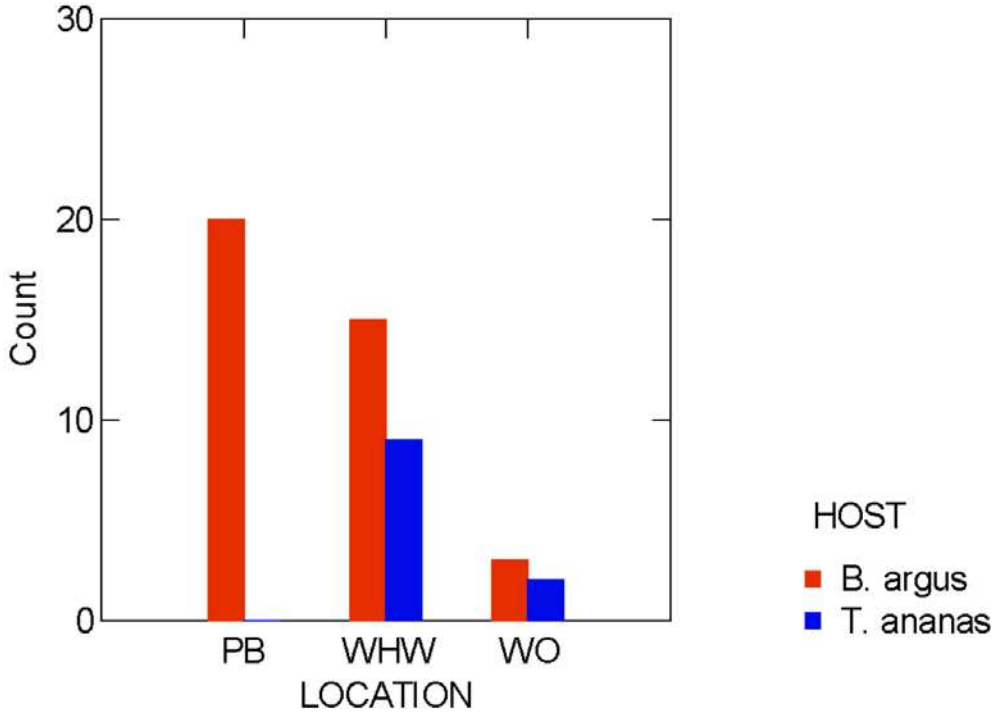


Figure 2: Carapidae species in relation to location

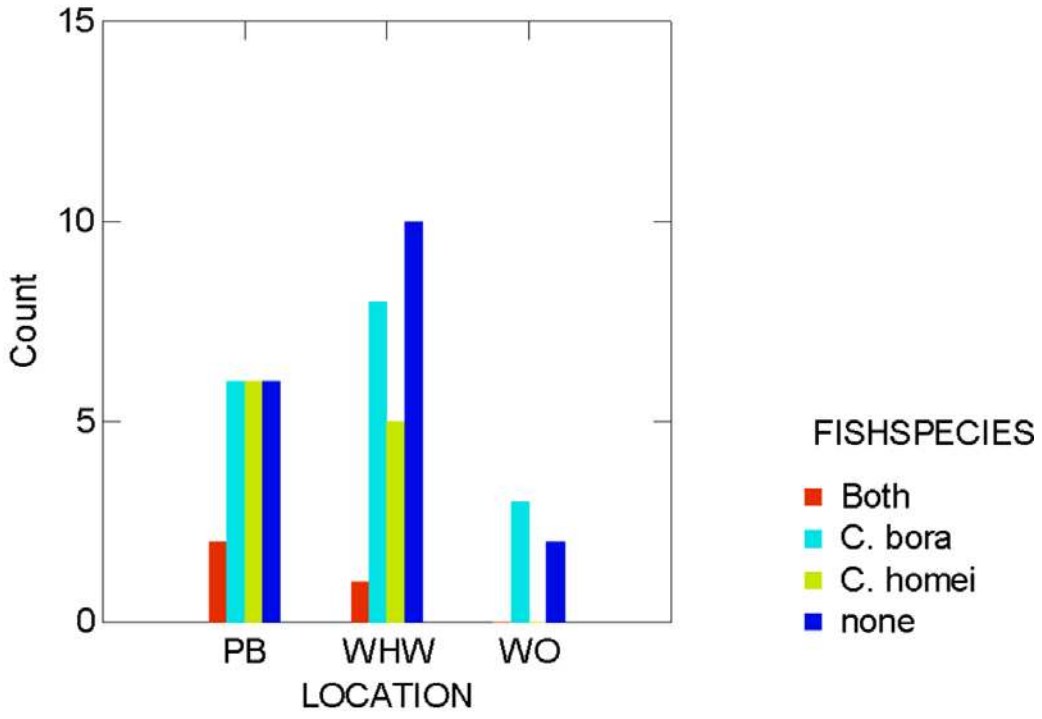
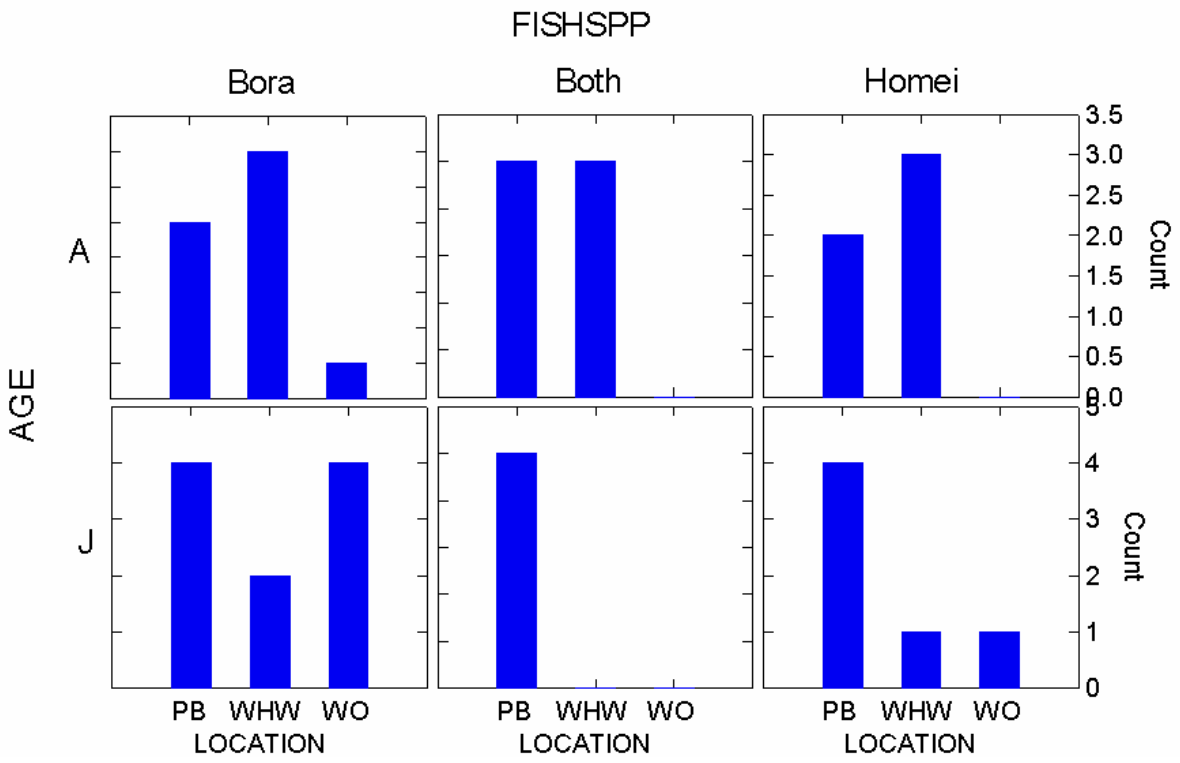


Figure 3: Carapidae species and age with relation to location



gure 4: Host color morphology in relation to Carapidae species