Title
HOST PREFERENCE, DETECTION, AND DEPENDENCE: THE ECTOPARASITIC GASTROPODS MELANELLA ACICULA AND PEASISTILIFER NITIDULA (EULIMIDAE) ON HOLOTHURIAN HOSTS

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HOST PREFERENCE, DETECTION, AND DEPENDENCE: THE ECTOPARASITIC GASTROPODS *MELANELLA ACICULA* AND *PEASISTILIFER NITIDULA* (EULIMIDAE) ON HOLOTHURIAN HOSTS

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Abstract. Parasites are ecologically significant organisms and must be understood to properly appreciate nearly any community. Parasitism is one of the most common (if not *the* most common) lifestyles, and parasites can influence species throughout a community. One group of parasites, the Eulimidae, is a large family of marine gastropods. Unfortunately, eulimids have not been thoroughly studied and host use behaviors have not been well characterized at the specific, or even generic levels. Therefore, this study seeks to describe host preference, host detection and tracking, and dependence on host access for two eulimid species, both sharing the macrohabitat environment. A series of experiments and a field survey showed that *Peasistilifer nitidula* was host specific, actively located hosts by chemical cues, reattached to hosts quickly, and required frequent access to the host for survival. Conversely, *Melanella acicula* had a preferred host but parasitized others as well, did not actively pursue hosts by chemical or visual detection methods, reattached infrequently in the short-term, and could survive longer isolated from the host. Using these aspects of host use to compare these co-existing species showed significantly different life histories, and suggests possible niche differentiation between a generalist and specialist species.

Keywords: parasite, sea cucumber, Holothuroidea, snail, chemosensory, niche

INTRODUCTION

Parasitism is a prevalent feeding habit (Lafferty et. al. 2006, Price 1977, Renaud 1996) and an important biotic interaction. Parasitic species have implications for the life history of hosts, competing parasitic species, and consequently the overall community structure (Combes 1996, Lafferty et. al. 2006, Rea 1994, Poulin 1999, Thomas 2000, Wood et. al. 2007). Lyskin (2005) asserts that symbioses (and therefore, parasitisms) are good systems through which to view biological interaction, as the host dampens the effect of abiotic factors on the symbiont - making biological interactions more clear to scientific inquiry.

The parasitic lifestyle can require unique adaptations and specializations, morphologically and in aspects of life history (Poulin 1963, Warén 1983) - and lead to extensive adaptive radiation (Price 1977). Furthermore, evolution of parasitic species can be driven by the pressures of this lifestyle, and evolution of host species can be accelerated by the influences of the parasite, i.e. coevolution (Combes 1996, Poulin 1963, Renaud 1996, Warén 1983).

To further understand the parasitic system, a case study of parasite-host interactions was organized on the French Polynesian tropical island of Mo'orea. Mo'orea is a relatively young member (1.5-2 million years old) of the

Society Islands, a volcanic chain formed by hot-spot action. These islands are relatively remote, being over 5000km away from Australia, the nearest mainland.

Eulimidae, a family of parasitic marine gastropods, were the focus of this study, specifically the tropical shallow-water species *Melanella acicula* Gould and *Peasistilifer nitidula* Pease. The Eulimidae is a large family, estimated to be the second largest family of parasitic gastropods (Lozouet 2001, Warén 1983) and also showing the greatest diversity and highest level of morphological adaptation (Warén 1983). There have been 1250 species described as of 1983, although this includes fossil species and species that are likely synonymous. However, there are numerous undescribed species and many more expected to be found as well (Warén 1983). A recent taxonomic revision by Bouchet and Rocroi (2005) place the eulimid superfamily, Eulimoidea, in the informal group Ptenoglossa with two other superfamilies (Epitonioidea and Triphoroidea). The Ptenoglossa along with sister taxa, the Neogastropoda and Littorinimorpha, compose the Hypsogastropoda - including animals such as periwinkles, cowries, worm snails and numerous predatory snails (Bouchet and Rocroi 2005). More specific documentation of the natural history and taxonomy of the Eulimidae is underdeveloped (McClain 2006, Warén pers. comm.); as such, a study of host-parasite interactions will be doubly useful, allowing general insights into parasite systems and increasing the knowledge base of eulimid natural history.

Most eulimids are ectoparasitic, feeding on the bodily fluids of echinoderm hosts by piercing the skin of the host and reaching an internal organ with their proboscis (Crossland 1991, Crossland and Warén 1991, Warén 1980-A, 1983, 1984). Some primitive forms retain a radula (Anonymous 1982), however *Melanella* and *Peasistilifer* have proboscises. *Melanella acicula* and *Peasistilifer nitidula* can be found parasitizing sea cucumbers (Class: Holothuroidae). Both *P. nitidula* and *M. acicula* insert their proboscis into the lacunae of the host's body wall, not fully penetrating the body wall, and feed upon the coelomocytes (blood and immune cells) (Hetzel 1963, Warén 1980-B, 1983).

Some eulimids are known to be host specific, others are more generalist (Crossland 1993). However, documentation of host use is not substantial enough to make general conclusions about the host specificity in Eulimidae – although genera tend to be specific to hosts at least at the class level (Warén 1983). As such, more thorough investigations, as in this study, will be useful. *Melanella* is known to parasitize holothurians, and *M. acicula* has been recorded on three host species other than those found during this project on Mo'orea (Warén 1983). *Peasistilifer nitidula* has been noted to be exclusively parasitizing the sea cucumber *Holothuria atra* (Warén pers comm.), and the occurrence of *P. nitidula* on *H. atra* can been seen in the literature (Warén 1980-B, 1983). However, I have not seen an experimental confirmation of the parasite's specificity.

Melanella acicula and *Peasistilifer nitidula* are both common and overlapping in distribution at the study sites for this project; a comparison of host use can show similarities and differences in life histories of these two organisms presented with the same problem: maximizing fitness as an ectoparasite of holothurians. Specifically, I wished to answer what hosts are being used, and how those hosts are being located. Dimensions of the host-parasite relationship that were characterized to answer these questions were: distribution in nature, host preference, host detection, and survival off the host. A field survey helped establish what species are parasitized in nature and the preference trials further checked for these host preferences under controlled conditions. Warén (1983) suggests that host specificity may be an important tool in approaching eulimid taxonomy, with more primitive species being less host specific, and more derived forms becoming increasingly specific. Host

detection trials included both chemosensory and visually based tests. Chemosensory abilities of various invertebrate parasites and predators have been recorded as a way to detect prey and hosts (Cervo 1996, Haas 1994, Rae 2009, Svensen 2000), and are hypothesized to be utilized by *M. acicula* and *P. nitidula*. The majority of eulimids have eyes with lenses, even species that are endoparasitic and/or abyssal (Warén 1983), and visual detection was tested as a possible host detection method. Survival time off the host allowed trends from other experiments to be correlated to dependency on the host; survivorship off the host for *P. nitidula* and *M. acicula* has been anecdotally noted as a "long time" (Warén 1983).

Holothurians are substrate sifting or filter feeding echinoderms, having the most diverse symbiotic assemblages in Echinodermata (Lyskin 2005) and serving as hosts to *M. acicula* and *P. nitidula*. Echinoderms are often considered to be good hosts, by virtue of their long lifespan and predator deterring abilities – creating a stable and protected environment for the symbiont (Warén 1983). Holothurians in particular show a number of predator defense mechanisms: expulsion of Cuvierian tubules, active avoidance, crypsis, unpalatbility, toxicity, thickening of the body wall, or evisceration of internal organs (defense by autotomy) (Castillo 2006). Three shallow-water species from the family Holothuriidae were used throughout the study as potential hosts and sources of parasites: *Bohadschia argus* Jäger, *Bohadschia marmorata* Jäger, and *Holothuria atra* Jäger. These host species were chosen for ease of collection, being more closely related to each other than other holothurians found, and having the highest parasite infection levels. Other sea cucumbers are considered briefly in the survey to better understand the distribution of *M. acicula* and *P. nitidula*.

MATERIALS AND METHODS

Study Sites

Field and lab components of this study were undertaken on Mo'orea from 12 Oct to 14 Nov 2009. Two sites were used as the main sources for all study organisms, the Richard *B.* Gump South Pacific Island Research Station located along Cook's Bay, and the Public Beach of Opunohu Bay. These adjacent bays open to the North, and are major features of the Mo'orean landscape (Figure 1). Temae Public Beach was also visited once for collecting.

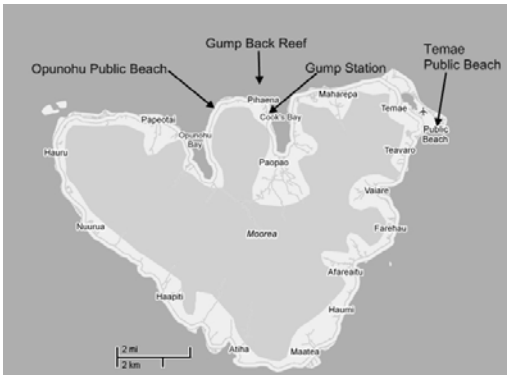

Figure 1. Map of Mo'orea Study Sites. (Google Maps 2009, edited)

Gump Station (17°29'25.96"S, 149°49'34.17"W) borders a marine protected area (i.e., a no-take zone), and serves as a research and boarding facility for various scientific research groups. Animals were collected within the reef flat area, up to 50.4m out from shore and at depths up to 2.7m. The reef flat substrate was a mix of sand and coral rubble, with algae encrusted rocks and coral heads scattered throughout. Additionally, a single trip was taken to the back reef (17º28' 57.48"S, 149º49'46"W), just outside of the marine protected area boundary. This area was mostly open and sandy, littered with coral rubble and dotted with a few large coral heads.

Opunohu Public Beach (17º29'25.31"S, 149º51'0.25"W) is a white-sand beach with minimal development (e.g. picnic tables), but

an appreciable amount of people traffic. Collecting was also done within the reef flat zone, with a maximum distance from shore of 76m and as deep as 7.5m. The coral and rock structure was generally more dense than at Gump Station, and a greater proportion of the coral was alive.

Temae Public Beach (17°29'50.61"S, 149°45'28.99"W) was similar to Opunohu in habitat, but with a longer stretch of open sand leading from shore before coral became a marked component of the fauna.

Collection of Organisms

Three holothurian species, *Bohadschia argus*, *Bohadschia marmorata*, and *Holothuria atra*, were used throughout this study as sources of eulimids and as hosts in experiments. These species were chosen for their ubiquity and abundance at the study sites, higher parasite loads, and grouping within the same family. Collection of *B. argus* and *H. atra* was done during the day or night as needed, *B. marmorata* was found only at night. Other holothuroian species found (*Synapta maculata* Chamisso and Eysenhardt, *Thelenota ananas* Jäger, *Stichopus horrens* Selenka) were checked in the water for *M. acicula* or *P. nitidula*, but no snails were collected; this was done only to roughly document the occurrence of the studied eulimds on hosts other than the three focused on. For all sea cucumbers, the method of collection was by hand while snorkeling. When a sea cucumber of interest was found, it was checked in the water for eulimids. If at least one *Melanella acicula* or *Peasistilifer nitidula* was found, the host was placed in a Ziploc plastic bag (one *Bohadschia* per bag, multiple *Holothuria* per bag) and carried in a plastic bin. The Ziploc bag and bin helped to minimize chances of snails washing off or moving between host species. Also, the containers reduced agitation by handling and the Ziploc bags contained cuvierian tubules expelled by *Bohadschia*.

Bohadschia argus, *B. marmorata*, and *H. atra* brought to shore were then processed for their eulimid parasites. The snails were removed by squeezing the surrounding tissue to push out the parasite, or forceps were used to pull the snail off. A eulimid expert, Anders Warén, made initial identifications for me, afterward, I made identifications by appraisal with the naked eye (color and shape were distinct between the species). Collected snails were sorted by species and by host species, and then used the same day in experiments.

Experimental Designs

Four experiments were run: (1) host preference, (2) chemical host detection, (3) visual host detection and (4) separation from the host. Trials did not use a standardized number of snails, the number run through a trial depended on the number collected that day. The two types of snails used in experiments were: *M. acicula* collected off *B. argus*, and *P. nitidula* off *H. atra*. Other snail types (snail species – host species combinations) were found in low numbers, and insufficient time was available to run all snail types through experiments.

Host Preference

All host preference trials were run using one individual of each of the sea cucumber species, and one of the snail types used in this study. Of the three *B. argus* color-morphs, the more common of the two dark morphs was used. One sea cucumber was placed in each of three corners of an outdoor tank (80cm x 50cm x 40cm) with the fourth corner containing a drain pipe to move water out of the tank (Figure 2). Snails were placed in the middle of the tank, and thereafter all organisms were allowed to move freely. A slow inflow of seawater into the tank was run throughout the trial. Any eulimids found floating at the water's surface were pushed back down. And, conspicuous animals other

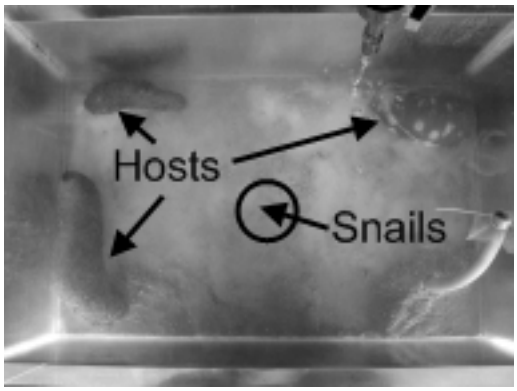

Figure 2. Host Preference Tank Setup.

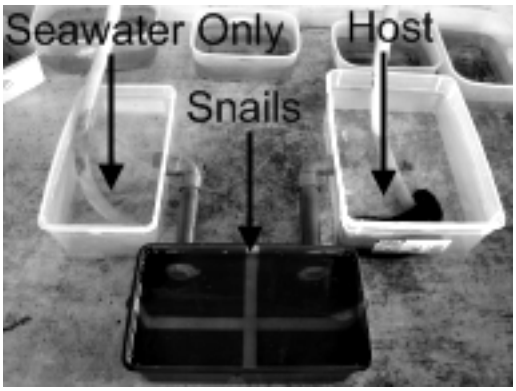

Figure 3. Chemical Host Detection Tank.

than the experimental organisms were removed (e.g. other symbionts).

This design allowed infection trends from the field to be checked against a controlled situation – with no differences in host abundance, habitat, or interference from species other than desired experimental organisms.

The sea cucumbers were checked by hand for eulimids once a day, and the number of snails found on each host was recorded (regardless of whether the snail had fully reattached or was found crawling on the host). Any damage incurred to the snail by the removal process was assumed to regenerated by the snail in a short time (Warén, pers. comm.), and reattachment for these species has been documented previously (Warén 1983). Host preference trials were run for a week, or until all snails were on hosts. Four replicates were performed with *P. nitidula* (128 snails total) and five with *M. acicula* (69 snails). Between each replicate, the tanks were cleaned – debris scooped out and tank walls rinsed with seawater.

Chemical Host Detection

Snails were tested for chemosensory detection of hosts by exposing the parasites to simultaneous flows of seawater-only and seawater washed over a sea cucumber. The experimental setup consisted of a central box with two pipes leading in, each pipe also connected to another box (Figure 3). Each of these two other boxes had seawater flowing in and through to the central box. One of the boxes carrying water to the central box would contain a sea cucumber. Mesh covered the ends of the pipes leading into the central box as well as a drain hole in the wall opposite the pipes, and a lid was kept on the box between counts. The central box receiving water flow from the pipes was black to prevent snails from seeing the host, and was also demarcated to divide the box in fourths in order to quantify snail location. Snails detecting the presence of a host by chemical cues were expected to approach the corresponding pipe exit. Two sea cucumbers were used: *H. atra* when running *P. nitidula*, and *B. argus* for *M. acicula*. Control trials in which no host was present in the host-box were conducted to generate expected snail distribution frequencies.

Placing dye in the host-box and monitoring the flow of into the snail box showed a greater concentration of dye to be in the two quadrants on the side of the box with the host-box pipe, but the dye did spread throughout the box. The flow entering the central box had a marked pattern, spreading in sequence from the upper right quadrant to the lower right to the upper left and finally to the lower left (Figure 4).

Trials were run for 24-36 hours, with four snail counts during that period, with at least four hours between counts. The number of snails in each quadrant was tallied during these counts. Five with-host trials and three

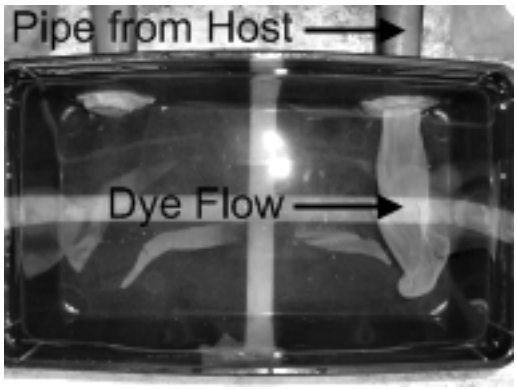

Figure 4. Beginning of dye flow into snail box.

control trials for both snail species were run. A total of 159 and 183 *P. nitidula* were used in with-host and control replicates respectively, and 64 (with-host) and 35 (control) *M. acicula* were tried. Similarly to host preference trials, the experimental setup was cleaned out with seawater between runs.

Visual Host Detection

Testing for possible visual acquisition of hosts, the snails were put in a tank divided by clear plastic walls – one chamber containing a host. Two walls were placed in the tank, creating three chambers: two 14cm x 24cm x 20cm chambers on the ends, and a 20cm x 24cm x 20cm chamber in between (Figure 5).

Snails were placed in the middle chamber, a potential host in one end chamber (with a drain pipe and water flow), and the other end chamber contained only seawater. The dividing walls were flush with the tank walls and had sealant applied to prevent water from one chamber moving into another – thereby avoiding possible transmission of chemical cues from the host. The walls of the main tank were an opaque gray to exclude outside visual stimuli. If snails were visually acquiring hosts, then more snails were predicted to approach the wall dividing the host and middle chamber. The middle chamber was marked with a dividing center line running parallel to the clear walls – snails were counted as being

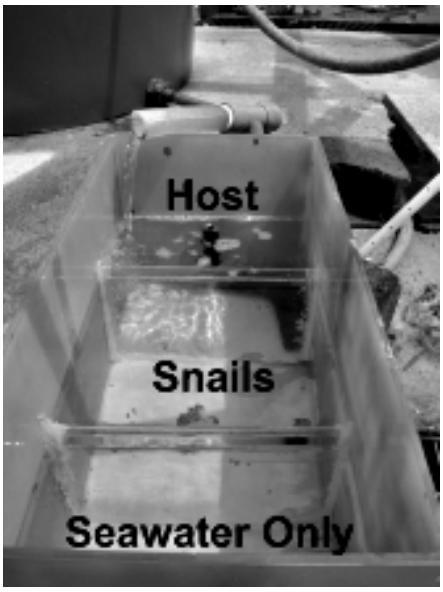

Figure 5. Visual Detection Tank.

on either the host-side or the blank-side of the middle chamber.

As with chemical detection trials, *H. atra* was used when running *P. nitidula*, and *B. argus* with *M. acicula*, as well as host-free control trials. Trials were conducted in a similar manner to chemosensory detection experiments, 24-36 hours were allotted per replicate, with checks spaced at least four hours apart. Three with-host trials for *P. nitidula* were conducted, all other trials had two replicates. A total of 58 *P. nitidula* and 33 *M. acicula* were used in with-host trials, 30 *P. nitidula* and 24 *M. acicula* for controls.

Separation from Host

Survivorship of snails removed from hosts was tested by storing isolated snails in vials with pierced tops and seawater flowing over them. This experiment lasted for 21 days, with 24 *P. nitidula* and 18 *M. acicula*. Daily counts were taken of snails alive and dead. Snails were determined to be dead if they did not move within 3 minutes of having their vial agitated.

STATISTICS

Experiment replicates did not have a standardized number of snails per trial, and I have assumed that snail density does not significantly affect the outcome of the experiments, and that all data can be combined for statistical analysis. Statistical tests were done using Microsoft Excel and NCSS 2007.

Host Preference

To track and plot reattachment over the 7 day period, an average value of snails reattached was calculated per day, for each snail specie and host specie combination.
Chi-square analysis was used to test *M. acicula* trends for significant deviation from expected values using the null hypothesis that all hosts are equally parasitized – predicting that each host would have 1/3 of the reattached parasites. Every count was added, giving the total number of snails found reattached throughout the study for each host species. These values represent the observed values, adding them and dividing by 3 yield the expected values.

Chemical and Visual Host Detection

Data from replicates of the same trial type (i.e., by snail species and with host or control) were combined to generate expected and observed frequencies of snail distribution by quadrant (chemosensory experiment) or tank side (visual experiment). Control trials gave expected frequencies, which were applied to the total number of snails counted in with-host trials to generate expected values for a chi-square analysis. The total number of snails counted was used for this calculation instead of total number of snails put into the experiment because some snails were not found (either escaping, or simply not found). The average number of snails per quadrant or tank side from with-host trials was calculated, and used as the observed value.

Separation

A log rank test was used to compare survivorship trends between *M. acicula* and *P. nitidula*. NCSS 2007 was used to run the test.

RESULTS

Collection

Melanella acicula was found on multiple host species: *H. atra*, *B. argus*, *B. marmorata*, and *T.. ananas*. However, *M. acicula* was found with the greatest abundance and frequency on *B. argus*. *Peasistilifer nitidula* was only on a single host species, *H. atra*. Data collected for the three hosts used in experiments always show a minimum of at least one snail and possibly inflated snail counts, this is because hosts of these species were only processed and recorded if at least one snail was found when searching in the field (Table 1). Certainly, individuals were found with no eulimids, although for *H. atra* this was uncommon.

Gump Station and Opunohu sites showed different abundances of *P. nitidula* and *M. acicula*. Gump showed generally higher

Table 1. Eulimid collection records for hosts species used in experiments. All sites combined.

Host Species	Number Collected	Proportion with *P. nitidula*	Average # of *P. nitidula*	Range of *P. nitidula*	Proportion with *M. acicula*	Average # of *M. acicula*	Range of *M. acicula*
B. argus	94	0	0	0-0	1	2.73	1 - 14
B. marmorata	12	0	0	0-0	1	1.75	1 - 4
H. atra	73	.96	7.48	0-43	.27	0.60	0 - 8

counts of *M. acicula*, having average counts of 1.7 (*H. atra*) and 3.1 (*B. argus*), and Opunohu with 0.14 (*H. atra*) and 1.7 (*B. argus*). Conversely, Opunohu yielded higher *P. nitidula* counts on *H. atra*: 7.9 (Opunohu) and 3.8 (Gump).

No eulmids were found on *S. maculata*, and one *S. horrens* was found with eight *Melanella*, but the species was unclear. Snail counts on *T.. ananas* ranged from 0-2 *M. acicula*, most of which with 0. Eulimids of other species were also found on *B. argus* and *T.. ananas*, some of which were a *Melanella* species other than *M. acicula*. Numbers of *S. horrens* and *S. maculata* found were small, *T.. ananas* was found in greater abundance: 4 *S. horrens*, 3 *S. maculata*, and 14 *T.. ananas*.

Host Preference

Despite overall low reattachment levels for *M. acicula*, a noticeably greater number were found on *B. argus* (Figure 6); chi-square analysis reported that *M. acicula* re-infection patterns differ significantly from an equal distribution across the hosts (p = 1.04E-08). With a much higher reattachment rate, *P. nitidula* showed a strong trend in reattaching, being found almost solely on *H. atra*. Fluctuations in reattachment trends are due to either snails moving off the host between checks, or my missing them during counts.

Chemical Detection of Host

A chi-square analysis of snail distribution among the quadrants shows a significant deviation from expected values generated by control trials for *P. nitidula* (p = 1.89E-08), however this not so for *M. acicula* (p = 0.15). For *P. nitidula* there is a marked difference between the quadrants on the host flow side and water-only side, Figure 7 shows the numbering and layout of the quadrants. Frequencies of *M. acicula* are more even across the quadrants (Figure 8).

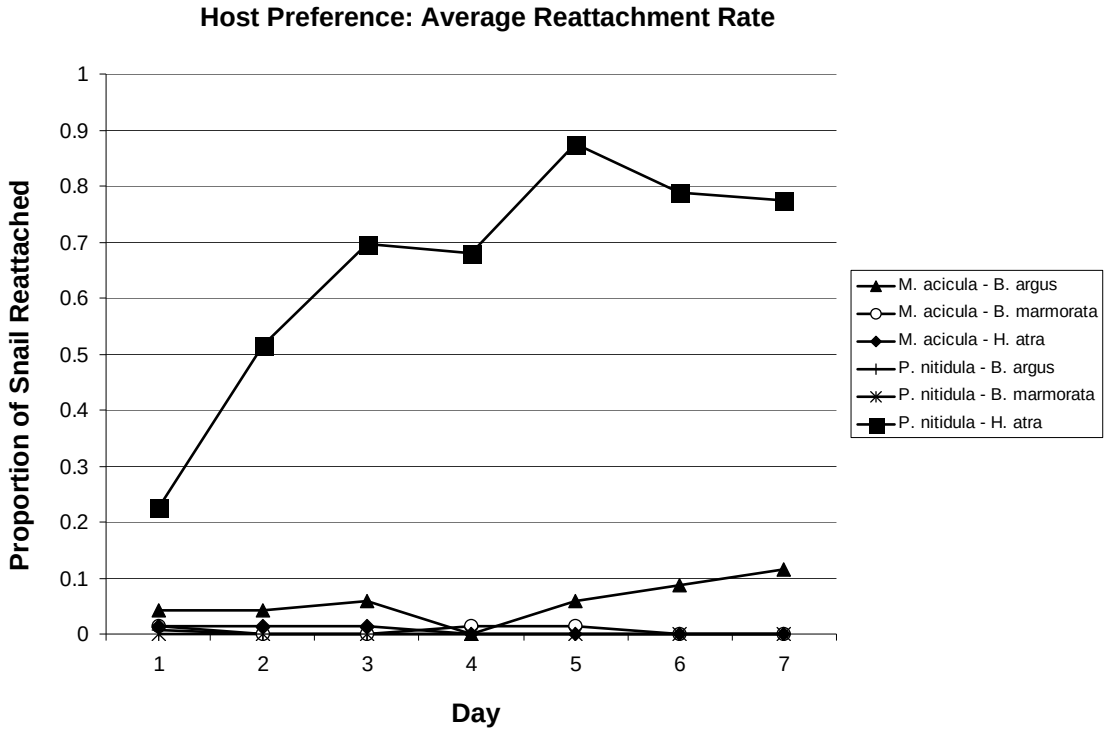

Figure 6. Snail reattachment rate by host species

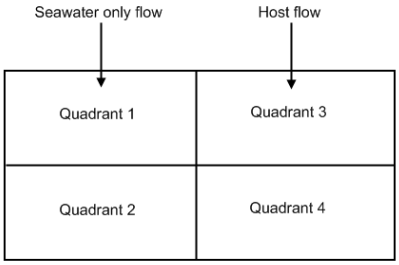

Figure 7. Chemical Host Detection Quadrant Map.

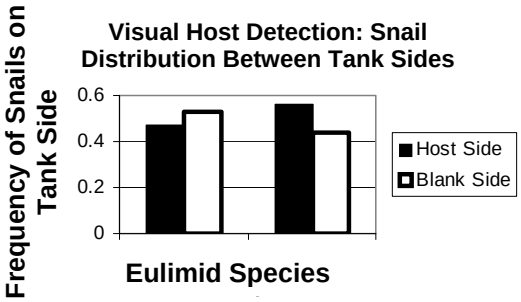

Figure 9. Snail distribution in visual detection trials

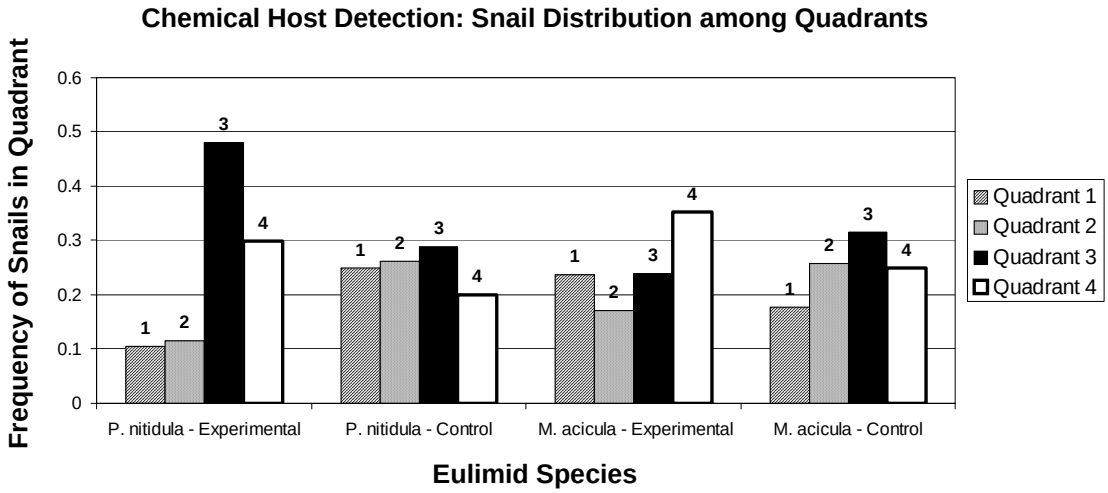

Figure 8. Snail distribution in chemosensory trials. Numbers above bars represent quadrant number.

Visual Detection of Host

Chi-square analysis comparing expected distributions of snails between the host and blank sides of the tank against observed results show non-significant results for both *P. nitidula* ($p = 0.23$) and *M. acicula* ($p = 0.22$). The snails were found in close to equal frequency on either side of the tank (Figure 9).

Separation from Host

Mortality levels and rate for *P. nitidula* after separation from the host were notably higher than in *M. acicula* (Figure 10). The two species were shown to have significantly different survival trends, as analyzed by a log rank test ($p = 0.0000$, NCSS only reports p-values to 4 decimal places).

DISCUSSION

Collection

The use of multiple host species has been documented for *Melanella* (Warén 1983), and the field data collected continues to support this – but also shows a bias for parasitizing *B. argus*. Finding *M. acicula* on four different host species, with members from two different

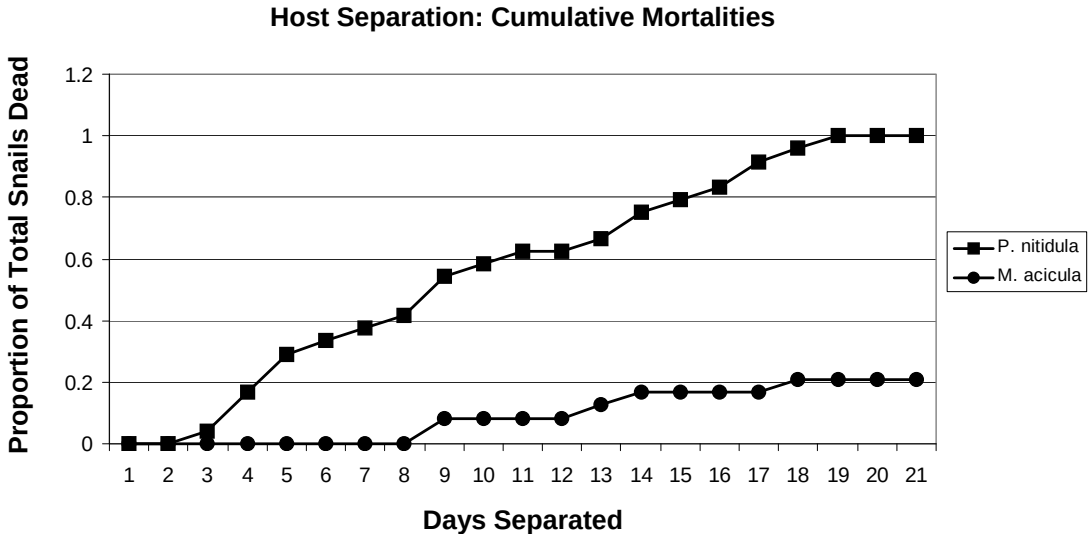

Figure 10. Deaths after separation from host. 24 *P. nitidula* and 18 *M. acicula*.

families, indicates that this eulimid is not highly host specific, but *B. argus* was found to have markedly more *M. acicula*, and this finding points to some degree of host preference. *Peasistilifer nitidula* was found exclusively on *H. atra*, this finding clearly supports the hypothesis that *P. nitidula* has a strong host preference.

Although, macrohabitat (habitat of the host) has been documented as a factor in parasite distribution (Rohde 1984, 1994) and appears to influence the occurrence *M. acicula* and *P. nitidula* . Gump Station had generally higher *M. acicula* counts on both *B. argus* and *H. atra*, and a greater proportion of *B. argus* to *H. atra* when compared to Opunohu. The converse is true for Opunohu and *P. nitidula* – a larger *P. nitidula* population, and fewer *B. argus* in comparison to *H. atra*. However, even at Gump, *B. argus* still showed the highest *M. acicula* infection levels, and at Opunohu, *P. nitidula* only parastized *H. atra* despite a much greater population density. Eulimid infection of *B. marmorata* was found to be similar between the two sites, but with only six infected individuals from either location, any conclusions are tentative. Although macrohabitat can affect abundance, general preference trends remain similar across habitats, appearing to indicate that other factors are significant in host selection.

The low numbers of *S. horrens* and *S. maculata* are in part due to these holothurians' nocturnal habit (thereby making collecting more difficult), and what seemed to be an overall scarcity compared to species like *B. argus* and *H. atra*. Additionally, the field survey portion of this study was introduced late and considered a less important component of the study. Because of these low counts, nothing meaningful can be said about the occurrence of *M. acicula* or *P. nitidula* on *S. horrens* and *S. maculata*. But, having found *M. acicula* on *T.. ananas* does show that host preference trials did not address all hosts used by this parasite.

Host Preference

Results in the lab support findings in the field – *P. nitidula* exclusively parasitizing *H. atra* and *M. acicula* parasitizing multiple species, but generally favoring *B. argus*. The experimental setup standardized abiotic and biotic factors, leaving the eulimids with only a choice of hosts. The three hosts were of different sizes, in order from largest to smallest: *B. argus*, *B. marmorata*, *H. atra*. As such, the possibility exists that larger hosts

were more often encountered or more easily detected, making the results reflect not only preference, but ease of access to the host. However, on the modestly sized floor space of the tank, this factor is not likely significant, if it is influencing results at all.

The low reattachment rates and levels of *M. acicula* are curious, as I would have expected a parasite to quickly reacquire a host after removal, as *P. nitidula* did. There are some possible explanations: the proboscis is likely damaged when the snail is removed while feeding (Warén pers. comm.) and perhaps a week is insufficient for a full regeneration of the organ, the snail may have trouble properly finding a host either due to poor detection abilities or confusion from the presence of three potential hosts in a single tank, or the *M. acicula* may not be pressured to find a new host within a one week time span.

Dogiel (1964) makes a general division of hosts in relation to the parasite, there being main, secondary, and accidental hosts. A number of factors are used to distinguish these categories, for example, parasites of main hosts are the most prevalent, reproductively successful, quickest maturing, and best adapted to their host; parasites on accidental hosts are rare and tend to show great difficulty in proper development and survival. Secondary hosts are a middle ground, parasites are less common on these hosts and face some difficulties in maximizing their use of the host (Dogiel 1964). This division of hosts may serve well to characterize the relationship of *M. acicula* to its hosts, *B. argus* being its main and preferred host, while other species such as *B. marmorata* and *H. atra* could be secondary hosts. Further studies that describe the fecundity and quality of *M. acicula* found on these various hosts would serve to better understand the parasite's relationship to its hosts and ecological niche.

To understand host specificity of a parasite is to understand, in part, the habitat of that parasite – Rohde (1994) suggests that host specificity as one of several key characters in defining the ecological niche of a parasite. However, the characterization of host specificity is a complex problem and is not fully understood (Rohde 1984). Factors involved in the development of host preference may include: morphological or physiological factors, availability of potential hosts in time and space, behaviors of the host, and macrohabitat (Rohde 1984, 1994). Interestingly, with little deviation seen in lab from natural trends, ecological factors such as those listed may be playing a minimal role in host selection for the studied organisms.

Chemical Host Detection

The tendency for *P. nitidula* to congregate near the host-scented water flow indicates that chemical cues are used in host detection. The design of the experiment prevented direct physical and visual contact with the host, leaving only chemical cues as an indication of the host's presence. Quadrant 3 had greater snail counts than quadrant 4 (the two quadrants with the greatest level of host flow), this makes sense given that quadrant 3 was nearest the input of host water flow. And, both of these quadrants had markedly higher snail levels than quadrants 1 and 2, the quadrants facing the input of only water. This distribution among the quadrants supports the hypothesis that these snails are chemically orienting to hosts.

However, *M. acicula* did not show this trend, rather, there was no significant difference between trials of water only and trials with a host. This finding would seem to signify two possibilities: *M. acicula* cannot detect chemical cues from potential hosts, or that *M. acicula* is not actively pursuing hosts regardless of detection. This study cannot give a good answer for which of these two possibilities is more likely.

The trends observed in both *P. nitidula* and *M. acicula* line up well with findings from host preference trials that show a much higher rate and level of infection by *P. nitidula* than *M. acicula*. From the chemical host detection trials *P. nitidula* demonstrated the use of

chemosensory abilities by actively moving toward host flow, and in host preference trials, *P. nitidula* showed corresponding rapid and thorough infection *H. atra*. On the other hand, *M. acicula* displayed no difference in behavior when presented with flow containing host chemical cues, and preference trials reflect this in low reattachment rate and level.

Host detection is considered an important aspect of parasite biology, influencing the evolution of life history and interactions between host and parasite (Rea and Irwin 1994). Actively locating and infecting a host may require the parasite to respond to: environmental cues that direct it toward appropriate habitat, cues from the host's "active space" (the space the host modifies with its presence: e.g., by shadows, turbulence, heat), and ultimately, direct contact with the host itself (MacInnis 1976, Rea and Irwin 1994). The chemical detection displayed by *P. nitidula* is an example of response to the active space of the host. And, the correlation between this behavior and other dimensions of host use serves as a possible demonstration of the tie between the development of life history and host detection.

Visual Host Detection

Neither eulimid specie showed a significant difference in tank distribution from controls in visual detection tests; from this finding, the conclusion that vision is not used in tracking hosts can be drawn. Because *P. nitidula* showed a marked attraction to hosts in both preference and chemical trials, I assume that if *P. nitidula* could identify a host visually, the snails would move toward it. But, again, for *M. acicula* there cannot not be a definite conclusion that these snails are not using this detection strategy; they may be demonstrating a disinterest in hosts detected, rather than not being able to see their hosts. I should note that there were fewer replicates conducted than would have been preferred, and this deficit of trials may weaken conclusions formed from the collected data.

Separation from Host

Separation trials tested how long a snail could survive when isolated, without any host to parasitize. *Peasistilifer nitidula* showed a higher death rate – mortalities beginning sooner and increasing more quickly than *M. acicula*. This increased dependence on the host may help to explain observations made in other experiments. As *P. nitidula* showed a greater need for its host than *M. acicula* in the short-term (the separation experiment lasted 21 days), the behavior of approaching water with host chemical cues and more vigorously infecting hosts can be viewed as a reflection of this dependency. Similarly, with *M. acicula* showing both higher survivorship when separated and less activity in host detection and infection, one may draw the conclusion that *M. acicula* does not require as frequent access to its hosts – and correspondingly did not seek to reattach.

Conclusions

Various dimensions of the relationship of parasite and host have been characterized, and can be brought together to better understand the system as a whole. The observations indicate that a more generalist strategy is used by *M. acicula* and that *P. nitidula* is more of a specialist. Despite, or perhaps more appropriately, because, their shared habitat and seemingly similar lifestyle (ectoparasites of shallow water holothurians) these two organisms show markedly different approaches to host use. These findings begin to show how niche partitioning may be occurring between these species and the corresponding differences in life history of a more generalist versus a more specialist parasite. In short, *P. nitidula* demonstrates a high degree of host specificity for and reliance on *H. atra*, actively seeking out their host by chemosensory mechanisms. *Melanella acicula* shows a different pattern; it parasitizes multiple holothurian species (showing

preference for *B. argus*) and is able to survive longer away from these hosts, and as such does not begin to seek hosts to reattach to shortly after being removed.

Although aspects of host use would seem to be a logical starting point in understanding the life history of a parasitic organism, there has been relatively little work done to describe eulimid behavior toward hosts. Additionally, as holothurians and other echinoderms may be considered ecologically important due to their strong contribution to the biomass of benthic communities (Jangoux 1984), parasitic organisms interacting with them have a corresponding importance. Therefore, better understanding host use patterns by the eulimids represent an important step in further understanding the ecology of an important, large, and understudied group.

Future Studies

The findings in this study are only a beginning to understanding the behavior and life history of *M. acicula* and *P. nitidula* – observing the patterns described in this paper naturally lead to questions seeking to understand how, and why. There is an apparent host specificity or preference in these snails, why is that? Possible avenues of investigation could look into structural differences in the proboscis between the snails, and relate that to differences in the body wall of the hosts. Or, more physiological rather anatomical differences could be studied, for example testing snail resistance to toxins from different sea cucumber species. As the *M. acicula* used in preference trials were only from *B. argus*, a useful study would be to check host species fidelity – i.e., are individuals removed from *B. argus* more likely to reattach to *B. argus* than those removed from *B. marmorata* or *H. atra*. Alternately, investigations of host preference could be taken to an interspecific rather intraspecific scale. The parasites may have the ability to discriminate hosts depending on factors other than species, such as size, color, age, health, preexisting parasite load, etc.

Another interesting question is what chemical cues is *P. nitidula* detecting from their hosts, some sea cucumbers use pheromone communication (Hamel and Mercier 1996) that may be detected by the parasite, and at what range or density can kairomones be identified by the parasite is worth testing. And, if *M. acicula* is deprived of host access longer, will it too begin to actively seek its host?

Although no notable effects of parasite infection were seen, studying the affect of parasite load on the host would still be a meaningful project. A focused study may pick up on effects too subtle or long-term for my casual observation to elucidate.

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