Settlement and Metamorphosis Preferences of Larvae of the Corallivorous Nudibranch *Phestilla melanobrachia.*

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INTRODUCTION

Many benthic marine invertebrates have a planktonic larval stage in which the larvae live and feed in the water column before settlement. Often the larvae must find a particular substrate or host on which to settle, and use chemical cues to do so, although little is known about the nature of these chemical cues (Pawlik, 1992). This type of settlement, which has been termed associative settlement (Crisp, 1974), is found in cases where larvae are settling on a food, host, or symbiont.

At least three species of the nudibranch *Phestilla* live as well as feed on corals, and thus fall into this category of settling on food or host (Rudman, 1981). As might be expected, the larvae of *Phestilla* use some cue to locate and settle on host corals in the field (Harris 1975, Hadfield and Scheuer 1985). *Phestilla sibogae* has been widely studied, and as a result, much is known about its larval ecology. *Phestilla sibogae* lives on corals of the genus *Porites*, and will not feed on other corals even when starving (Harris 1971). Phestilla sibogae larvae are positively phototactic initially, but lose this phototaxis when metamorphically competent (Miller and Hadfield, 1986). Once competent, *P.sibogae* larvae settle and metamorphose in response to a water-borne chemical cue released by their host corals (Hadfield and Schueur 1985, Hadfield and Pennington 1990). Both coral polyps and "coral water" (water in which adult coral colonies have been soaked overnight) induce settlement and metamorphosis of *P. sibogae* (Hadfield and Pennington, 1990). This cue can cause reversible habituation if introduced to the larvae before competence (Hadfield 1984). Non food-source corals do not induce metamorphosis, but some organic solvents (Pennington and Hadfield 1989), neuroactive compounds (Hadfield 1984, Hirata and Hadfield 1986, Hadfield and Scheuer 1985), and

well as excess potassium (Yool et al. 1986; Hadfield and Pennington 1990, Pechenik et al. 1995) can induce metamorphosis The rhinopores of the adult nudibranchs appear to be the main chemosensory receptor for the coral cue (Murphy and Hadfield, 1997), whereas the apical sensory organ appears to be the receptor for the larvae (Hadfield et al. 2000). In addition the central nervous system of the larvae has been implicated in mediating the process of settlement and metamorphosis (Pires and Hadfield 1991, Pires and Hadfield 1993, Kempf et al. 1992).

In contrast to this bank of knowledge available for *Phestilla sibogae*, available knowledge on *Phestilla melanobrachia* is sorely lacking. The objective of this study is to learn more about the specificity of metamorphic processes of *Phestilla melanobrachia*, specifically how larvae respond to chemical cues released by host corals.

WHAT IS KNOWN ABOUT *Phestilla melanobrachia*?

Adult *Phestilla melanobrachia* live in close proximity to dendrophyllid corals, relying on them for food as well as protective camoflauge. Adult *P. melanobrachia* on Guam are found living on or next to corals of the genus *Tubastrea*, and perhaps other members of the dendrophyllid family. The coloration of the adult nudibranch resembles that of an individual coral polyp, and thus *P. melanobrachia* is able to avoid detection when on or next to a coral colony. *Phestilla melanobrachia* in Hawaii form relationships with dendrophyllid corals other than *Tubastrea*, and take on the coloration of whatever host coral is being fed upon. Thus *P. melanobrachia* living on *Tubastrea spp* is reddish-orange with yellow serrata, while *P. melanobrachia* living on *Turbinaria spp.* is white with black serrata (Harris 1971). Diet-derived pigments sequestered in the body facilitate this camoflauge, and also allow a fairly quick window for adaptation (Harris 1973). *P.*

melanobrachia taken from one host coral and placed on another change color patterns within a week.

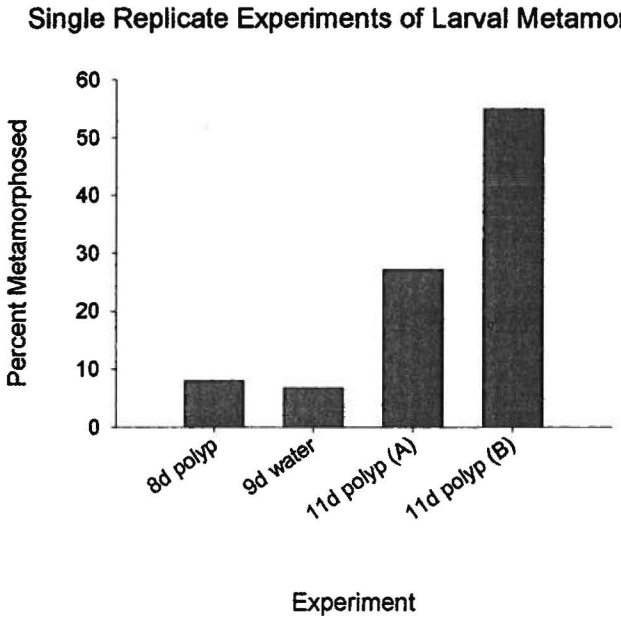
Phestilla melanobrachia larvae are planktotrophic veligers, and do not attain competence for at least 8 or 9 days after hatching. Tests by Harris show that 8-10 day old *P. melanobrachia* larvae will settle and metamorphose in the presence of host coral fragments, but not in plain seawater (Harris 1975). He reported that veligers would settle on or near the polyp, and would shed their shells in 24-48 hours. The percent metamorphosing in response to coral varied from 0-13.4% during the course of his experiments. Based on these results by Harris, I ran some preliminary tests on settlement and metamorphosis of *Phestilla melanobrachia* larvae.

PRELIMINARY TESTS AND RESULTS:

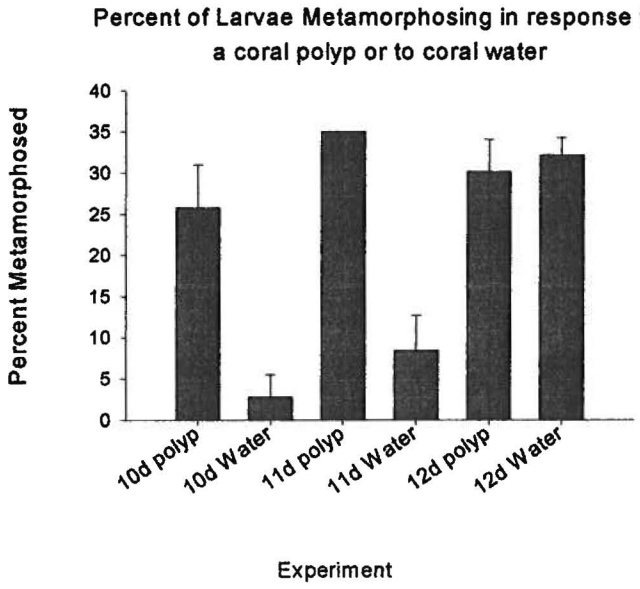
I collected adult *P. melanobrachia* and *Tubastrea sp.* from Apra Harbor, Guam. Adults and corals were maintained in an outdoor tank, and the nudibranchs used the corals for food as well as a place to lay egg masses. Egg masses were collected 5 days after deposition and artificially hatched in petri dishes by ripping the egg cases with a pair of fine forceps. Veligers were immediately transferred from the petri dishes to 1L beakers filled with antibiotic seawater (6μg/ml Penicillin G and 5μg/ml streptomycin sulfate) and 5 mL of *Isochrysus galbana* ($3x10^4$ cells/mL Tahitian strain). Beakers were rigged to keep a constant flow of water dripping onto the surface to reduce larvae being caught in the surface tension. Larvae were cultured at densities of ~1000 larvae per beaker (1 larvae/mL).

For settlement assays, between 20-100 larvae were transferred to individual 5ml beaker cups, and the exact number of larvae per cup recorded. Treatment (coral polyp, coral water, or seawater) was added to the beaker cups, and the cups were checked 24 hours later to look for juvenile *Phestilla* that had shed their shells and metamorphosed. Cups were checked again 48 hours after treatment for settlement and metamorphosis, and again after 72 hours. Larvae not metamorphosed after 72 hours were considered unaffected by the treatment.

Coral water was prepared by soaking pieces of live coral in aerated, filtered seawater overnight (18hours), and then filtering the water through a coffee filter or millepore filter to remove large particles from the water. The first tests I did were to look at the effects of coral polyps and coral water on competent larvae. The tests I conducted were single replicate, and results are shown in the graph below:



Percent metamorphosis for 8-day old larvae was under 10% when exposed to a coral polyp, and also under 10% for 9-day old larvae exposed to coral water. Control treatments (filtered seawater) consistently showed no metamorphosis, and are not included on the graph. The 8-day old larvae took 48 hours to metamorphose. However, 11-day old larvae showed a higher percent metamorphosis, and completed the metamorphic process in under 24 hours. Based on these results I set up a test with multiple replicates. Results are shown below:



Percent metamorphosis was higher in the polyp treatment than the coral water treatment for 10 and 11-day old larvae. The metamorphic process took under 24 hours, and the negative control treatment (filtered seawater, not shown on graph) showed no metamorphosis. However, 12-day old larvae responded equally to both polyp and coral water treatments, again with the negative control showing no metamorphosis.

PROPOSED TESTS:

Based on the results of these preliminary tests, I propose the following:

Hypothesis #1: Larvae increase in competence from days 7-8 through days 13-14, reaching a point where % metamorphosis no longer increases, but stays constant. Larvae of age 8-10 days will settle at higher rates when exposed to coral polyps as opposed to coral water, but at equal rates in the two treatments when older (11-14 days).

I propose to test this hypothesis in the following manner:

Test #1: I will conduct metamorphosis assays on larvae from the same batch sequentially starting from day 6 using both coral polyp and coral water, and continuing past day 12 until competence declines or an asymptote is reached. Metamorphosis assays will consist of 25 larvae per 5 mL beaker cup, 10 beaker cups per treatment. Larvae will be transferred to the beaker cups by plastic pipette, and treatment will be added within a few minutes of transfer. Treatments will be as follows:

A. Filtered *Tubastrea* water

B. *Tubastrea* polyp

C. Filtered seawater (control)

The beaker cups will be checked for metamorphosed animals after 24 hours, and again after 48 hours. Larvae will be counted as metamorphosed when the shell has been completely shed and the metamorphic process completed. Percent metamorphosed will be calculated as:

$$\frac{\text{Number metamorphosed}}{\text{Total number tested}} * (100)$$

For each treatment, the percent metamorphosed will be averaged for all ten replicates, and the standard deviation and standard error calculated. Assuming that the filtered seawater control will yield close to zero percent metamorphosis, a 2-way ANOVA will be performed on the averaged means for *Tubastrea* water versus *Tubastrea* polyp. Factors tested will be age and treatment. Bar graphs similar to those in the Preliminary Results section will be constructed using mean and standard error for treatments across all days tested, i.e. day 6 larvae through day 14/15 larvae (last day yet to be determined, see above).

My second objective is to determine which corals are used as food and host by adult and larval *P. melanobrachia*:

Hypothesis #2: Larvae will settle and metamorphose in response to polyps and coral water from food-source corals but not in response to other non-food corals.

Test #2a: To determine which corals are preferred as food, and if any non-preferred corals will be consumed when no other alternatives are present, choice assays will be performed with adult *Phestilla*. Choice assays will be conducted by placing two different species of coral in a small basin of running seawater, and adding an adult *Phestilla* to the basin. Ten adults will be simultaneously tested in separate basins.

The adults will be watched over the next 2 hours, and position recorded every 10 minutes. Proportion of time spent on each coral will be calculated as:

$$\frac{\text{Number of times scored on coral}}{\text{12 total scorings}}$$

Coral species to be tested will include all dendrophyllid corals from Guam, as well as some other common coral species such as *Porites rus*, particularly corals used by other species of *Phestilla*. Proportion of time spent on each coral species will be summarized in a table format, and analyzed using a one-way anova to determine if there are differences in time spent on each substrate.

Test #2b: Metamorphosis assays will be conducted on larvae at various stages of competence (as determined in test #1) using food and non-food corals (as determined in test #2a). Metamorphosis assays will be run in the same manner as test #1, with treatments being:

A. Coral water

B. Coral polyp

C. Filtered seawater (control)

My third objective deals with a result seen by Harris (1971) in which *P. melanobrachia* veligers were preferentially attracted to coral colonies occupied by *P. melanobrachia* adults. Harris (1973) also observed that adult *Phestilla* are often seen in gregarious clusters in the field. It is possible that veligers are using a cue from conspecific adults to preferentially guide settlement. It is also possible that attacked corals are releasing a cue that is attracting the veligers to settle. Or there may be some interaction between adult and prey that is attracting veligers.

Hypothesis #3: Larvae are preferentially attracted to coral polyps on which an adult is feeding over lone coral polyps.

Test #3: Metamorphosis assays will be conducted on competent larvae in the same manner as test #1. Treatments will be as follows:

A. Control – filtered seawater
B. Coral water
C. Coral + adult water (adults feed on live coral in aerated beaker for 18-hour period, then water is filtered and prepared the same as coral water. Multiple adults used)
D. Polyp
E. Partially eaten polyp
F. Polyp + adult
G. Adult alone
H. Adult water (adults left in aerated water for 18-hour period, then water filtered and prepared the same as coral water).

As with test #1, percent metamorphosis will be determined for each replicate, and averaged across treatments. A one-way ANOVA will be run on the data, with a Tukey's comparison across all treatments.

In addition to the three tests proposed above, I also have some general goals and tests to run based on results of tests 1-3:

-Determine polarity, MW, and some general characteristics of the chemical cue by using bioassay-guided fractionation of the compound.

-If hypothesis #3 is accepted, determine polarity, MW, and some general characteristics of the adult+coral cue using bioassay-guided fractionation of the compound.

The extent to which these tests are pursued will be highly dependent on the level of difficulty required to isolate the compound(s) in question.

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