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Larval metamorphosis of *Phestilla* spp. in response to waterborne cues from corals

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ABSTRACT

Many marine invertebrates depend on their larvae for dispersal and to settle and metamorphose in the appropriate habitat for adult survival, yet the mechanisms of habitat selection remain poorly understood. In Hawaii, the nudibranch *Phestilla sibogae* only feeds on *Porites compressa* and requires a water-soluble cue from this coral for metamorphosis. On Guam, we tested three different species of *Phestilla* to determine if their larvae require water-soluble compounds from corals to induce metamorphosis. Larvae of *P. sibogae* metamorphosed at high rates to waterborne cues from multiple species of corals in the genus *Porites*. Larvae of *Phestilla minor* could distinguish among waterborne compounds from different species of *Porites*, but also had high rates of metamorphosis in filtered seawater and in response to corals that adults did not eat. Larvae of *Phestilla* sp. 2 could distinguish among water-soluble cues from different species of *Goniopora* and consistently had the highest rates of metamorphosis in response to waterborne cues released from *Goniopora fruticosa*. *P. minor* was the only species studied that did not require waterborne cues for larval metamorphosis.

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1. Introduction

Larval recruitment is a critical process that can determine the structure of marine communities (Caley et al., 1996; Gaines and Roughgarden, 1985; Grosberg and Levitan, 1992; Roughgarden et al., 1988; Underwood and Fairweather, 1989). Marine invertebrate larvae can use physical, chemical and biological cues to choose appropriate settlement substrata (Pawlik, 1992; Scheltema, 1974; Zimmer and Butman, 2000). Specialist predators need to find their prey species for survival, and in some cases their larvae have been shown to choose the appropriate settlement substrates for adult survival (Pawlik, 1992). Larvae of specialist predators often use chemical signals from their host to find the appropriate habitat and stop their dispersal phase, termed settlement. Some larvae also require chemical cues to induce metamorphosis, which is the physiological and morphological transformation from a larva to a juvenile.

Larvae of many marine molluscs can settle and metamorphose in response to chemical cues released by their prey, including sea hares (Switzer-Dunlap and Hadfield, 1977), the queen conch *Strombus gigas* (Boettcher and Targett, 1996), the dorid nudibranch *Adalaria proxima* (Lambert and Todd, 1994; Lambert et al., 1997), and the sacoglossan *Alderia modesta* (Krug, 2001; Krug and Manzi, 1999; Krug and Zimmer, 2000). *Phestilla sibogae* is a specialist aeolid nudibranch that has been studied for its larval ecology, physiology and behavior during the last 30 years (Hadfield, 1977; Hadfield and Paul, 2001). Larvae of *P. sibogae* in Hawaii metamorphose in response to a water-soluble cue released from the host coral *Porites compressa* (Hadfield and Scheuer, 1985; Hadfield and Pennington, 1990). Larvae of *P. sibogae* are capable of detecting the inductive cue in the water column, and in the presence of the inducer they stop swimming and settle to the benthos (Hadfield and Koehl, 2004). A better understanding of larval behavior has led to the development of a model which predicts the spatial patterns of recruitment of individual larvae (Koehl et al., 2007), the conclusions of which were validated by field surveys for recruitment of *P. sibogae* (Hadfield et al., 2006). Even though the larval ecology of *P. sibogae* has been well studied, the larval behavior and ecology of other species in this genus have only recently been studied.

On the tropical Pacific island of Guam, five genetically distinct species of nudibranchs in the genus *Phestilla* were found, each with different host specificity and life history characteristics (Faucci et al., 2007; Ritson-Williams et al., 2003, 2007). How do these differences in life history characteristics influence the larval ecology of these specialist predators? In this study we compared three species of *Phestilla* to determine if they all metamorphosed in response to water-soluble cues from their preferred prey and tested the diversity of coral species that produce metamorphic inducers.

2. Materials and methods

2.1. Species studied

P. sibogae (Bergh, 1905) is an aeolid nudibranch that feeds on at least six species of corals in the genus *Porites* on Guam (Ritson-Williams et al., 2003). *P. sibogae* has been synonymized under the

Abbreviation: FSW, 0.2 μm filtered seawater.

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name *P. lugubris* (Rudman, 1981); however, to be consistent with a large body of literature we use the name *P. sibogae*. Larvae of *P. sibogae* are known to metamorphose in the presence of water-soluble compounds from *P. compressa* in Hawaii and will metamorphose in response to other corals in the genus *Porites*. Larvae of *P. sibogae* will also metamorphose in response to some coral species it will not eat, but at lower rates than in response to corals of the genus *Porites* (Ritson-Williams et al., 2003).

P. minor (Rudman, 1981) is a small (max. 7 mm) aeolid nudibranch that feeds on *Porites annae* and *Porites lutea* on Guam (Ritson-Williams et al., 2003). The larvae of *P. minor* can be competent for metamorphosis as soon as or before 1 day after hatching, and previous experiments showed that approximately 10 to 20% of the larvae can spontaneously metamorphose in 0.2 μm filtered seawater (FSW) (Ritson-Williams et al., 2007).

Phestilla sp. 2 is morphologically similar to *P. sibogae* but is genetically distinct (Faucci et al., 2007). Adults of *P.* sp. 2 feed on some but not all species of corals in the genus *Goniopora* on Guam and Palau (Ritson-Williams et al., 2003). Larvae of *P.* sp. 2 are lecithotrophic and are competent for metamorphosis approximately 4 days after hatching (Ritson-Williams et al., 2007).

2.2. Larval cultures

Adult nudibranchs were collected from the field and maintained in the laboratory in flow-through seawater aquaria on their prey corals. *P. sibogae* was fed *Porites* (*Synaraea*) *rus*, *P. minor* was fed *P. annae*, and *P.* sp. 2 was fed *Goniopora fruticosa*. Egg masses from each species of *Phestilla* were collected daily and maintained in larval chambers with daily changes of 0.2 μm filtered seawater (FSW) as described in Ritson-Williams et al. (2003). When the veliger larvae were fully formed and moving (typically 5 days after fertilization), they were hatched from multiple (5–10) egg masses and placed in antibiotic FSW (90 μg ml^{-1} penicillin G and 75 μg ml^{-1} streptomycin sulfate) in two to four larval chambers. The larval chambers were surrounded by flow-through seawater to maintain ambient seawater temperatures (approximately 28–32 °C), and antibiotic FSW was exchanged from the larval chambers daily. After hatching, larvae were maintained without feeding in antibiotic FSW in these chambers until they were competent for metamorphosis, which was 4 days for *P. sibogae* and *P.* sp. 2 and 1 day for *P. minor*.

2.3. Preparation of waterborne cues

For the larvae of *P. sibogae* a variety of coral species in the genus *Porites* were tested to determine if there was a difference in metamorphic inducers for as many species of *Porites* as possible. For the larvae of *P.* sp. 2 initial experiments showed that these larvae would respond to a water-soluble metamorphic inducer from *G. fruticosa*. Subsequent experiments tested coral water prepared from different species of *Goniopora* to determine if water-soluble metamorphic inducers were found in the corals that *P.* sp. 2 was known to feed on (Ritson-Williams et al., 2003) and at what dilutions waterborne cues from these corals could induce metamorphosis. Initial experiments with larvae of *P. minor* showed no difference in the inductive activity of water-soluble cues from multiple corals species. *P. minor* feeds preferentially on two corals in the genus *Porites*; therefore, water-soluble cues from its preferred prey *P. annae* were compared to water-soluble cues from corals that it would not feed on (Ritson-Williams et al., 2003) to determine if larvae of *P. minor* would metamorphose at the same rates to waterborne cues from preferred and non preferred prey.

All of the coral fragments (approximately 2 cm long) were broken off of their colony at least 24 h before their use in an experiment or for preparing coral waterborne cues. They were held in flow-through seawater to allow them to recover from the fragmentation. Waterborne cues (hereafter termed "coral water") were prepared by soaking fragments of each species of coral in seawater. Fragments of the corals were placed as densely as possible in a glass beaker with seawater and aerated with an air stone for 24 h. Coral water conditioned with *P. compressa* was made with the same methods during a visit to Hawaii. Corals in the genus *Goniopora* were conditioned in seawater for 48 h to prepare coral water because the 24 hour incubations did not induce metamorphosis in *Phestilla* sp. 2 (Ritson-Williams et al., 2007). After 24 or 48 h, the conditioned coral water was decanted from the coral fragments and filtered through a paper coffee filter. The coral water was then frozen for use in subsequent experiments to prevent decomposition of active components. Before the coral water was added to any bioassay it was thawed to room temperature and then added to the larval experiments. The coral water prepared in this manner was considered 100% in the dilution assays and was combined with FSW at 1:1 and 1:3 volumes for the 50% and 25% concentrations.

2.4. Larval bioassays

Metamorphosis bioassays were conducted to determine the amount of larval metamorphosis in response to a variety of treatments. Ten to fifty larvae were placed in individual 5 or 9 ml wells of Costar® media culture well plates (nos. 3513 and 3516). The larvae were placed in each well in as little filtered seawater as possible (approximately 0.3 ml) to minimize the dilution of the coral water treatments. After the addition of the larvae the wells were filled to 5 ml with one of the following treatments: FSW, a coral fragment

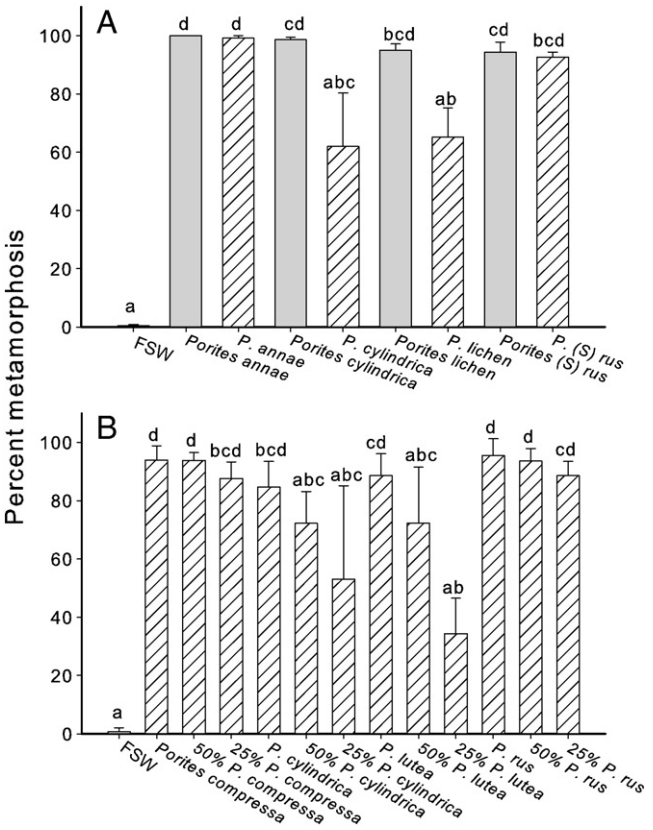

Fig. 1. Larval metamorphosis of *P. sibogae*. All larvae were tested 4 days after hatching and scored after 24 h of exposure to the treatments. Bars represent the mean percentage of larval metamorphosis, and error bars are +1 SE. The same letters above the bars indicate means that are not significantly different as determined by Tukey's post-hoc test. A. Larval metamorphosis in response to filtered seawater (FSW), coral fragments (shaded bars) or coral conditioned water (striped bars). There were five replicates and the ANOVA results were $F = 10.33$ and $p < 0.001$. B. Larval metamorphosis in response to various dilutions of coral conditioned water. There were four replicates and the ANOVA results were $F = 9.99$ and $p < 0.001$.

(with FSW to fill the volume of the well), or coral water. At least four replicate well plates were used for each experiment as is indicated in the figures. In each well the numbers of remaining veligers and empty shells (the juvenile slugs are transparent and difficult to count accurately) were counted after exposure to the treatments for 24 h for *P. sibogae* and 48 h for *P. minor* and *P.* sp. 2. The percent metamorphosis for each replicate was calculated as the number of empty shells divided by the total number of larvae added to the well multiplied by 100. Figures show untransformed data.

For each species, the data (proportion metamorphosis) were arcsin square-root transformed and analyzed with a one-way ANOVA. If the arcsin square-root transformed data did not meet the assumptions of ANOVA the data were rank transformed and then analyzed with a one-way ANOVA. The results of each ANOVA are described in the figures. Each ANOVA was followed by a Tukey's post-hoc test to determine significant groupings. All analyses were done with Statistix 7 (Analytical Software).

3. Results

Larvae of *P. sibogae* had more metamorphosis in response to the fragments of *Porites* spp. and their coral water than in response to FSW (Fig. 1A). When tested with coral water made from *P. annae*, *Porites cylindrica*, *P. lichen*, and *P. rus*, larvae of *P. sibogae* had the same amount of metamorphosis in response to waterborne cues as in response to the fragments of different *Porites* spp. (Tukey's post-hoc test). The full strength coral water of *P. compressa*, *P. cylindrica*, *P. lutea* and *P. rus* induced significantly more metamorphosis than FSW (Fig. 1B). Except for *P. lutea* at 25% concentration, there was no difference in the rates of metamorphosis among the full strength and subsequent dilutions of coral water, indicating that the larvae can detect waterborne cues from these corals at reduced concentrations.

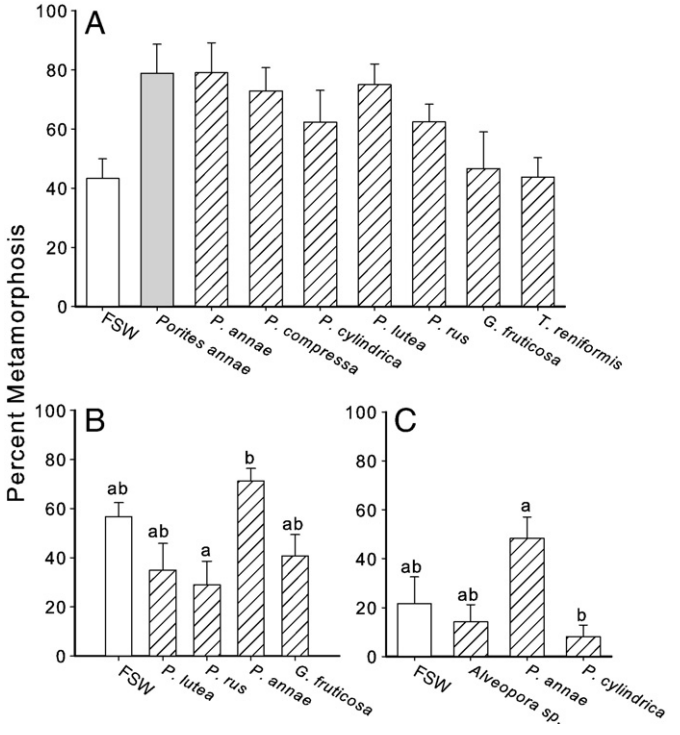

Fig. 2. Larval metamorphosis of *P. minor*. All larvae were tested 1 day after hatching and scored after 48 h of exposure to the treatments. Bars represent the mean percentage of larval metamorphosis, and error bars are +1 SE. The same letters above the bars indicate means that are not significantly different as determined by Tukey's post-hoc test. A. Larval metamorphosis in response to filtered seawater (FSW), coral fragments (shaded bars) or coral conditioned water (striped bars). There were four replicates and the ANOVA results were $F=2.75$ and $p=0.0234$. B. Larval metamorphosis in response to water conditioned with various coral species. There were four replicates and the ANOVA results were $F=4.27$ and $p=0.0167$. C. Larval metamorphosis in response to water conditioned with various coral species. There were four replicates and the ANOVA results were $F=3.50$ and $p=0.0498$.

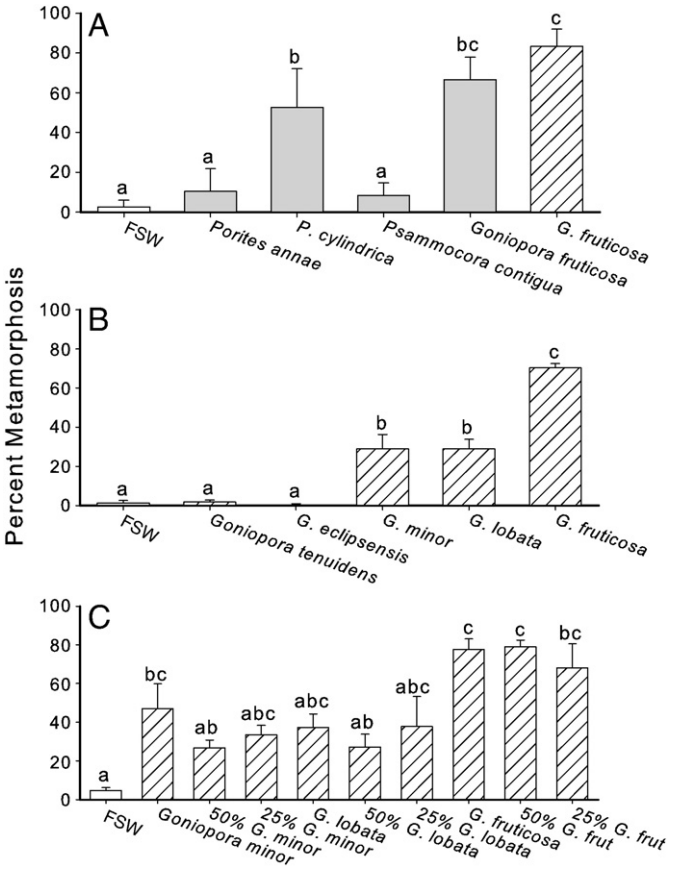

Fig. 3. Larval metamorphosis of *Phestilla* sp. 2. All larvae were tested 4 days after hatching and scored after 48 h of exposure to the treatments. Bars represent the mean percentage of larval metamorphosis, and error bars are +1 SE. The same letters above the bars indicate means that are not significantly different as determined by Tukey's post-hoc test. A. Larval metamorphosis in response to filtered seawater (FSW), coral fragments (shaded bars) or coral conditioned water (striped bars). There were seven replicates and the ANOVA results were $F=53.46$ and $p<0.001$. B. Larval metamorphosis in response to coral water conditioned with *Goniopora* spp. There were five replicates and the ANOVA results were $F=53.93$ and $p<0.001$. C. Larval metamorphosis in response to various dilutions of water conditioned with *Goniopora* spp. There were five replicates and the ANOVA results were $F=6.53$ and $p<0.001$.

Larvae of *P. minor* consistently had relatively high rates (20–60%) of metamorphosis in FSW during these bioassays (Fig. 2A, B, C). In a bioassay with fragments of *P. annae* (used as a positive control for metamorphosis), coral water made from a variety of coral species and FSW, larval metamorphosis in response to the various treatments differed (Fig. 2A), but Tukey's test did not discriminate among the treatments. In a second assay (Fig. 2B), the larvae of *P. minor* had higher rates of metamorphosis in response to coral water conditioned with *P. annae* (the preferred prey for adults) than to coral water from *Porites (Synaraea) rus*, but neither of these induced more metamorphosis than FSW (Tukey's post-hoc test). There were also significantly different rates of metamorphosis in response to coral water prepared from *P. annae*, which was greater than the metamorphosis in response to coral water from *P. cylindrica*, but again neither of these significantly differed from FSW (Fig. 2C).

Like *P. sibogae*, larvae of *Phestilla* sp. 2 did not metamorphose at high rates to FSW and required coral water-soluble cues for metamorphosis (Fig. 3A, B, C). *Phestilla* sp. 2 had more metamorphosis in response to fragments of *P. cylindrica*, *G. fruticosa* and the water-soluble cues from *G. fruticosa* than in response to FSW (Fig. 3A). There were different rates of metamorphosis in response to coral water

prepared from different corals in the genus *Goniopora* (Fig. 3B). Coral water from *Goniopora minor*, *G. lobata* and *G. fruticosa* induced more metamorphosis than FSW or coral water from *G. tenuidens* and *G. eclipsensis*, with the highest response to coral water from *G. fruticosa*. In the dilution assays, all of the dilutions of coral water induced the same amount of metamorphosis as the full strength coral water (Fig. 3C). Only the three concentrations of *G. fruticosa* coral water and the full strength *G. minor* coral water induced significantly more metamorphosis than FSW.

4. Discussion

In this study, the larvae of both *P. sibogae* and *Phestilla* sp. 2 required water-soluble cues released from their preferred prey for metamorphosis; however, *P. minor* did not require water-soluble cues for metamorphosis. *Phestilla melanobrachia* also metamorphosed in response to water-soluble cues released from its preferred prey, *Tubastraea aurea* (Ritson-Williams et al., 2007). Water-soluble cues are an important mechanism for pelagic larvae to detect their prey, mediating critical life history events such as settlement and metamorphosis. These experiments show that both *P. sibogae* and *P.* sp. 2 can metamorphose in response to multiple coral species and required water-soluble cues, not necessarily the coral itself, to induce metamorphosis.

P. sibogae is already well known to require a water-soluble cue from its host coral *P. compressa* for metamorphosis (Hadfield and Scheuer, 1985). This obligate relationship has been a valuable case study in larval ecology that has fostered a variety of interdisciplinary studies encompassing neurobiology (Kempf et al., 1992; Leise and Hadfield, 2000; Willows, 1985), larval behavior (Hadfield and Koehl, 2004; Miller and Hadfield, 1986), and modeling cue concentrations in relation to larval recruitment and transport (Hadfield et al., 2006; Koehl et al., 2007). The study presented here shows that multiple coral species in the genus *Porites* produce water-soluble compound(s) that induce metamorphosis in the larvae of *P. sibogae*. Larvae of *P. sibogae* can detect waterborne cues even at 25% concentrations that were tested during this study. The larvae of *P. sibogae* had similar rates of metamorphosis in response to all dilutions for most of the species of *Porites* studied (except for *P. lutea*). Research in Hawaii has shown that water collected from the reef induced the same amount of larval metamorphosis for *P. sibogae* as a 30% dilution of coral water from *P. compressa* prepared with the same methods as those used in this study (Hadfield and Koehl, 2004), suggesting that 25% is an ecologically relevant concentration for these experiments. Future experiments with field collected coral water would be an interesting comparison to our laboratory conditioned coral water. In the plankton, the larvae respond to inducer water by stopping their swimming and sinking to the benthos, which is probably a mechanism for larvae to settle adjacent to their host corals, ensuring adequate time for metamorphosis in a suitable habitat for growth and survival (Hadfield and Koehl, 2004). This is an immediate behavioral response to a short duration exposure to inducer water. A recent study showed that even a short exposure (2 h) to a metamorphic inducer was sufficient to induce settlement but not metamorphosis in the sacoglossan *Alderia* sp., and four day old larvae had higher rates of metamorphosis than one or two day old larvae in response to a 12 hour exposure to the inducer (Botello and Krug, 2006). A short term exposure of 2 h induced approximately 10% metamorphosis of larvae of *P. sibogae* after exposure to the metamorphic inducer from *P. compressa* (Hadfield, 1977). After 8 h of exposure to the inducer from *P. compressa* there was 30% metamorphosis and after 24 h there was 60% metamorphosis, showing that increased exposure time to the metamorphic inducer can increase rates of metamorphosis.

Phestilla sp. 2 also metamorphosed in response to waterborne cues from multiple corals in the genus *Goniopora*. The larvae of *P.* sp. 2 were selective and did not metamorphose in response to all of the *Goniopora* spp. tested, they only metamorphosed in response to the coral species that they would eat (Fig. 3B, (Ritson-Williams et al., 2003). In the field (on Guam) *P.* sp. 2 was only found on *G. fruticosa*, which was the only coral water that consistently induced more metamorphosis than FSW (Fig. 3A, B, C). These larvae had lower rates of metamorphosis in response to *Goniopora minor* and *G. lobata* (Fig. 3B). *Phestilla* sp. 2 can eat these two species, but was never found on them in the field (Ritson-Williams et al., 2003). Why *P.* sp. 2 does not eat all of the coral species in the genus *Goniopora* remains to be determined, but the larvae of *P.* sp. 2 consistently metamorphosed at the highest rates in response to the water-soluble compound(s) from its preferred prey, *G. fruticosa* (Fig. 3B, C). Seawater had to be incubated for 48 h with *Goniopora* spp. to create inductive coral water suggesting that these corals release less of the chemical cue(s) or that larvae of *P.* sp. 2 are less sensitive to inducer compounds than *P. sibogae*. Future experiments could test if larvae of *P.* sp. 2 respond to natural concentrations of the cue(s) collected from corals in the field and what duration of exposure to the cue(s) is necessary to induce larval metamorphosis.

Significant rates of metamorphosis in response to non-host corals were also observed. The larvae of *P.* sp. 2 metamorphosed at high rates in response to *P. cylindrica*, which it will not eat. High rates of settlement and metamorphosis onto non preferred prey is unlikely to occur in the field (on Guam) as we never found an individual of *Phestilla* on a coral that it would not eat in the laboratory (Ritson-Williams et al., 2003). However, it is important to note that larval settlement does not perfectly match adult feeding preferences, and it may be that some metamorphosis can occur in poor habitats, but would be undetectable in the field if the juveniles crawled onto other coral species or had high rates of post-settlement mortality.

P. minor has a different larval strategy than the other species of *Phestilla* studied here. The larvae of *P. minor* has a much shorter metamorphic competence time (Ritson-Williams et al., 2007), and in the laboratory its veliger larvae were often observed crawling on the benthos instead of swimming in the water (RRW and SMS, pers. obs.). The larvae of *P. minor* had the highest metamorphosis in response to their preferred prey *P. annae*; however, metamorphosis consistently occurred in the FSW and in response to water-soluble cues from other corals. In two of the experiments (but not the third) larvae of *P. minor* could distinguish between the water-soluble cues from their preferred coral (*P. annae*) and those of corals they would not eat such as *Porites rus* and *P. cylindrica* (Ritson-Williams et al., 2003). Since *P. minor* larvae have a short planktonic period and probably remain in the same habitat as their parents, settlement cues may be less important for finding suitable settlement substrata. The spontaneous metamorphosis in FSW observed in all of these assays (ranging from 20 to 60%) shows that these larvae have more flexibility in their metamorphosis than the other species of *Phestilla* studied here. This flexibility in metamorphosis has been described for other larvae (Hadfield and Strathmann, 1996), and might be a mechanism of host switching, which is a proposed process of diversification for *Phestilla* (Faucci et al., 2007).

There is a trend between diet breadth, larval competency time and the importance of water-soluble settlement cues in *Phestilla* spp. Previously, it was suggested that a greater diversity of prey corals is correlated with a longer planktonic phase (Ritson-Williams et al., 2007). This trend can also be linked to the diversity of corals that induce metamorphosis in these nudibranchs. Among *Phestilla* spp. only *P. minor*, and potentially *P.* sp. 1, do not require a waterborne metamorphic inducer. *P. minor* has the most restricted diet range and the shortest competency phase, all of which are strategies for recruiting into its natal habitat. Larvae of the sea hare *Aplysia californica* metamorphosed in response to a variety of macroalgal species but as juveniles would crawl onto preferred macroalgae (Pawlik, 1989). *A. californica* has planktotrophic larvae (that reach metamorphic competence 35 days after hatching), and larvae of

A. californica can metamorphose onto a variety of algal species after dispersal between habitats. This is a similar strategy as *P. melanobrachia* which eats corals in multiple genera and has planktotrophic larvae. Pawlik (1989) describes the trade off between settlement specificity and larval dispersal "…larvae appear to be selective enough to increase the likelihood of juvenile survival, but sufficiently broad in their response so as to minimize larval mortality." Some opisthobranchs have solved this trade off with a bet hedging strategy and have both lecithotrophic and planktotrophic larvae (Krug 2001; Smolensky et al., 2009). Settlement cues and metamorphic inducers are required for many marine larvae; however, even within the genus *Phestilla* there is a range of strategies for settling in the appropriate habitat for post-settlement survival.

Settlement cues for marine invertebrates have the potential to influence benthic community structure (Grosberg and Levitan, 1992); however, relatively few settlement cues have been isolated and characterized. Histamine was found to be the settlement inducer for the sea urchin *Holopneustes purpurascens* (Swanson et al., 2004). The red alga *Delisea pulchra* produced higher concentrations of histamine than the other algae tested, and histamine was released into the water surrounding the alga at high enough concentrations to induce settlement in some of the larvae (Swanson et al., 2006). The data presented here are a first step in understanding the chemical nature of the settlement cues for nudibranchs in the genus *Phestilla*. Even though there has been some recent progress on characterizing metamorphic inducers, water-soluble cues remain difficult to isolate and characterize (Hadfield and Paul, 2001; Paul and Ritson-Williams, 2008). These cues are often released at very low concentrations, which limits the material available for chemical characterization (Fine et al., 2006). Further work on isolation and characterization of the water-soluble cues that induce metamorphosis in *Phestilla* spp. will greatly increase our knowledge of how these nudibranchs find their host corals, and what types of compounds can influence larval settlement ecology. The data presented here show the importance of water-soluble cues from coral species that some, but not all, *Phestilla* spp. require for metamorphosis.

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References

Bergh, L.S.R., 1905. Die Opisthobranchiata der Siboga Expedition. Siboga-Expedite 50, 1–248.
Boettcher, A.A., Targett, N.M., 1996. Induction of metamorphosis in queen conch, *Strombus gigas* Linnaeus, larvae by cues associated with red algae from their nursery grounds. J. Exp. Mar. Biol. Ecol. 196, 29–52.
Botello, G., Krug, P.J., 2006. 'Desperate larvae' revisited: age, energy and experience affect sensitivity to settlement cues in larvae of the gastropod *Alderia* sp. Mar. Ecol. Prog. Ser. 312, 149–159.
Caley, M.J., Carr, M.H., Hixon, M.A., Hughes, T.P., Jones, G.P., Menge, B.A., 1996. Recruitment and the local dynamics of open marine populations. Annu. Rev. Ecol. Syst. 27, 477–500.
Faucci, A., Toonen, R.J., Hadfield, M.G., 2007. Host shift and speciation in a coral-feeding nudibranch. Proc. R. Soc. Lond. B - Biol. Sci. 274, 111–119.
Fine, J.M., Sisler, S.P., Vrieze, L.A., Swink, W.D., Sorensen, P.W., 2006. A practical method for obtaining useful quantities of pheromones from sea lamprey and other fishes for identification and control. J. Great Lakes Res. 32, 832–838.
Gaines, S., Roughgarden, J., 1985. Larval settlement rate — a leading determinant of structure in an ecological community of the marine intertidal zone. Proc. Natl. Acad. Sci. U. S. A. 82, 3707–3711.
Grosberg, R.K., Levitan, D.R., 1992. For adults only — supply-side ecology and the history of larval biology. Trends Ecol. Evol. 7, 130–133.
Hadfield, M.G., 1977. Chemical interactions in larval settling of a marine gastropod. In: Faulkner, D.J., Fenical, W.H. (Eds.), Marine Natural Products Chemistry. Plenum Press, New York, pp. 403–413.
Hadfield, M.G., Koehl, M.A.R., 2004. Rapid behavioral responses of an invertebrate larva to dissolved settlement cue. Biol. Bull. 207, 28–43.
Hadfield, M.G., Paul, V.J., 2001. Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock, J., Baker, B. (Eds.), Marine Chemical Ecology. CRC Press, Boca Raton, pp. 431–462.
Hadfield, M.G., Pennington, J.T., 1990. Nature of the metamorphic signal and its internal transduction in larvae of the nudibranch *Phestilla sibogae*. Bull. Mar. Sci. 46, 455–464.
Hadfield, M.G., Scheuer, D., 1985. Evidence for a soluble metamorphic inducer in Phestilla: ecological, chemical and biological data. Bull. Mar. Sci. 37, 556–566.
Hadfield, M.G., Strathmann, M.F., 1996. Variability, flexibility and plasticity in life histories of marine invertebrates. Oceanol. Acta 19, 323–334.
Hadfield, M.G., Faucci, A., Koehl, M.A.R., 2006. Measuring recruitment of minute larvae in a complex field environment: the corallivorous nudibranch *Phestilla sibogae* (Bergh). J. Exp. Mar. Biol. Ecol. 338, 57–72.
Kempf, S.C., Chun, G.V., Hadfield, M.G., 1992. An immunocytochemical search for potential neurotransmitters in larvae of *Phestilla sibogae* (Gastropoda, Opisthobranchia). Comp. Biochem. Physiol. C 101, 299–305.
Koehl, M.A.R., Strother, J.A., Reidenbach, M.A., Koseff, J.R., Hadfield, M.G., 2007. Individual-based model of larval transport to coral reefs in turbulent, wave-driven flow: behavioral responses to dissolved settlement inducer. Mar. Ecol. Prog. Ser. 335, 1–18.
Krug, P.J., 2001. Bet-hedging dispersal strategy of a specialist marine herbivore: a settlement dimorphism among sibling larvae of *Alderia modesta*. Mar. Ecol. Prog. Ser. 213, 177–192.
Krug, P.J., Manzi, A., 1999. Waterborne and surface-associated carbohydrates as settlement cues for larvae of the specialist marine herbivore *Alderia modesta*. Biol. Bull. 197, 94–103.
Krug, P.J., Zimmer, R.K., 2000. Larval settlement: chemical markers for tracing production, transport, and distribution of a waterborne cue. Mar. Ecol. Prog. Ser. 207, 283–296.
Lambert, W.J., Todd, C.D., 1994. Evidence for a water-borne cue inducing metamorphosis in the dorid nudibranch mollusk *Adalaria proxima* (Gastropoda, Nudibranchia). Mar. Biol. 120, 265–271.
Lambert, W.J., Todd, C.D., Hardege, J.D., 1997. Partial characterization and biological activity of a metamorphic inducer of the dorid nudibranch *Adalaria proxima* (Gastropoda: Nudibranchia). Invert. Biol. 116, 71–81.
Leise, E.M., Hadfield, M.G., 2000. An inducer of molluscan metamorphosis transforms activity patterns in a larval nervous system. Biol. Bull. 199, 241–250.
Miller, S.E., Hadfield, M.G., 1986. Ontogeny of phototaxis and metamorphic competence in larvae of the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia). J. Exp. Mar. Biol. Ecol. 97, 95–112.
Paul, V.J., Ritson-Williams, R., 2008. Marine chemical ecology. Nat. Prod. Rep. 25, 662–695.
Pawlik, J.R., 1989. Larvae of the sea hare Aplysia californica settle and metamorphose on an assortment of macroalgal species. Mar. Ecol. Prog. Ser. 51, 195–199.
Pawlik, J.R., 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr. Mar. Biol. 30, 273–335.
Ritson-Williams, R., Shjegstad, S.M., Paul, V.J., 2003. Host specificity of four corallivorous *Phestilla* nudibranchs (Gastropoda: Opisthobranchia). Mar. Ecol. Prog. Ser. 255, 207–218.
Ritson-Williams, R., Shjegstad, S.M., Paul, V.J., 2007. Larval metamorphic competence in four species of *Phestilla* (Gastropoda; Opisthobranchia). J. Exp. Mar. Biol. Ecol. 351, 160–167.
Roughgarden, J., Gaines, S., Possingham, H., 1988. Recruitment dynamics in complex life-cycles. Science 241, 1460–1466.
Rudman, W.B., 1981. Further studies on the anatomy and ecology of opisthobranch molluscs feeding on the scleractinian coral *Porites*. Zool. J. Linn. Soc.-Lond. 7, 373–412.
Scheltema, R.S., 1974. Biological interactions determining larval settlement of marine invertebrates. Thalass. Jugosl. 10, 263–296.
Smolensky, N., Romero, M.R., Krug, P.J., 2009. Evidence for costs of mating and self-fertilization in a simultaneous hermaphrodite with hypodermic insemination, the opisthobranch *Alderia willlowi*. Biol. Bull. 10, 188–199.
Swanson, R.L., de Nys, R., Huggett, M.J., Green, J.K., Steinberg, P.D., 2006. In situ quantification of a natural settlement cue and recruitment of the Australian sea urchin *Holopneustes purpurascens*. Mar. Ecol. Prog. Ser. 314, 1–14.
Swanson, R.L., Williamson, J.E., de Nys, R., Kumar, N., Bucknall, M.P., Steinberg, P.D., 2004. Induction of settlement of larvae of the sea urchin *Holopneustes purpurascens* by histamine from a host alga. Biol. Bull. 206, 161–172.
Switzer-Dunlap, M.F., Hadfield, M.G., 1977. Observations on development, larval growth and metamorphosis of four species of Aplysiidae (Gastropoda: Opisthobranchia) in laboratory culture. J. Exp. Mar. Biol. Ecol. 29, 245–261.
Underwood, A.J., Fairweather, P.G., 1989. Supply-side ecology and benthic marine assemblages. Trends Ecol. Evol. 4, 16–20.
Willows, A.O.D., 1985. Neural control of behavioral-responses in the nudibranch mollusk *Phestilla sibogae*. J. Neurobiol. 16, 157–170.
Zimmer, R., Butman, C., 2000. Chemical signaling processes in the marine environment. Biol. Bull. 198, 168–187.