

Extrinsic and intrinsic factors affecting the metamorphic rate of *Tripneustes gratilla* (Echinodermata: Echinoidea)

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ABSTRACT: Experiments were conducted to determine the effects of water conditioned with *Sargassum* (i.e. fresh and ground *Sargassum* and excreta of *Sargassum*-fed *Tripneustes gratilla*) — as well as the effects of adult sea urchins (i.e. *Salmacis sphaeroides* and *T. gratilla*), larval age and parentage — on the settlement and metamorphic rate of full-sibling batches of *T. gratilla* larvae. The majority of larvae (35 to 57 d old) settled within the first hour of the assays, but significantly more settled and metamorphosed completely in the conditioned water treatments than in the control (boiled filtered seawater). Completely metamorphosed larvae in the control treatment indicate that specific cues are not necessary to induce settlement and metamorphosis of competent larvae. However, habitat cues enhanced metamorphic rate significantly. Complete metamorphosis occurred earlier in *Sargassum* treatments, and the number of completely metamorphosed larvae was significantly higher in the ground *Sargassum* treatment. Observations of early juvenile aggregations on *Sargassum* further suggest that algal metabolites may enhance benthic recruitment. The sensitivity of sea urchin larvae to waterborne cues differed among larval age groups. Older batches of larvae metamorphosed faster, particularly in the ground *Sargassum* treatment, while the intermediate age group also had high metamorphic rates in both sea urchin treatments. There were significant differences in metamorphic rate among full sibling batches of larvae of the same age, as well as significant interaction between batch and treatment over time. Results indicate that differences in the age of competent larvae and genetic factors affect the ability of larvae to detect cues to initiate and complete metamorphosis.

KEY WORDS: Metamorphic rate · *Tripneustes gratilla* · Habitat-associated cues · Larval age · Familial variation

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INTRODUCTION

Settlement and metamorphosis are complex processes brought about by the interaction between biotic and abiotic factors operating at different scales, spatially and temporally (Rodríguez et al. 1993). At settlement, larvae actively explore the physical and chemical characteristics of potential substrata. In the absence of suitable or preferred substrata, larvae may resume swimming and return to the water column. Alternatively, larvae may respond to surface or waterborne cues and attach to the substrate and undergo metamorphosis, an irreversible process that indicates

the passage of the planktonic larva to a benthic juvenile (Hadfield et al. 2001). Induction and inhibition of settlement and metamorphosis by natural and artificial chemical cues have been demonstrated for many benthic marine invertebrates such as cnidarians, hydroids, polychaetes, bivalves, gastropods, opisthobranchs, barnacles, crabs, sipunculans, phoronids, sponges, bryozoans and ascidians (e.g. reviewed in Hadfield et al. 2001, Hadfield & Paul 2001, Bishop et al. 2006). Cues used were either derived from the natural substrata (i.e. inducers associated with microbial films, diatoms, conspecific adults and associated prey) or from artificial and/or chemical means such as

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choline derivatives, L-DOPA, gamma-aminobutyric acid (GABA) analogues and catecholamines (i.e. reviewed in Morse 1990, Pawlik 1992, Rodríguez et al. 1993). While induction of settlement and metamorphosis have been studied in various species of sea urchins (e.g. Cameron & Hinegardner 1974, Pearce & Scheibling 1990a, Gosselin & Jangoux 1996, Naidenko 1996, Williamson et al. 2000, Lamare & Barker 2001, Rahmani & Ueharai 2001, Takahashi et al. 2002, Huggett et al. 2006, Swanson et al. 2006, Dworjanyn & Pirozzi 2008), the majority of studies only investigated induction of settlement and did not quantify metamorphic success (i.e. larvae that completed metamorphosis into juveniles). Moreover, the effect of larval age and its interaction with habitat-associated cues has received little attention. Consistent with the expectations of the original desperate larva hypothesis (DLH), older larvae of the Australian sea urchin *Holopneustes purpurascens*, a specialized herbivore with lecithotrophic larvae, metamorphosed at lower concentrations of—and shorter exposure to—histamine produced by an alga (Swanson et al. 2007). Studies on sea urchins, which are generalists with planktotrophic larvae, may provide insights on the predictions of the modified DLH, which considers the effects of larval energy intake (feeding or non-feeding), planktonic mortality and post-settled habitat specificity (generalist and specialist) on decreasing selectivity with age (Elkin & Marshall 2007). There are also no studies that have investigated the effects of genetic or epigenetic factors on metamorphic competence in sea urchins as have been found in the tropical abalone *Haliotis asinina* (Jackson et al. 2005).

The sea urchin *Tripneustes gratilla* is a large tropical species widely distributed in the Indo-West Pacific region. It is an herbivore that feeds on a wide range of algae and other macrophytes. It can attain a size of up to 16 cm in diameter and a height of 6 cm (Lawrence & Agatsuma 2001). It has planktotrophic larvae with a variable larval period depending on temperature and food conditions (Shimabukuro 1991, Juinio-Meñez et al. 1998, Dworjanyn et al. 2007). The transition from the planktonic stage to the benthic stage is a critical period such that few larvae settle and metamorphose successfully. Because of this, the use of inducers to enhance settlement and metamorphosis has been investigated in the development of culture protocols for this species (Dworjanyn & Pirozzi 2008). Likewise, studies on settlement and metamorphosis of this species could provide insights on factors affecting benthic recruitment (Juinio-Meñez et al. 2008a).

In Bolinao, northwestern Philippines, *Tripneustes gratilla* is commonly found in seagrass beds on the reef flat area together with *Salmacis sphaeroides*, and on the reef crest where *Sargassum* spp. abound. Juvenile

and adult *T. gratilla* feed on *Sargassum* spp. (Bacolod & Dy 1986, Shimabukuro 1991, M. A. Juinio-Meñez pers. obs.). Cultured *T. gratilla* fed with *Sargassum* spp. have better egg quality and higher growth rates and gonad yield than those fed with seagrass *Thalassia hemprichii* (Bangi 2001, Juinio-Meñez et al. 2008b), indicating the high nutritional value of this seaweed. In the field, we have observed early juvenile *T. gratilla* clustered on the base of fronds and stipes of this seaweed. In the present study, we investigated the effects of larval age, parentage and water conditioned with conspecific and sympatric adult sea urchins (i.e. *T. gratilla* and *S. sphaeroides*) and different forms of *Sargassum* on settlement and metamorphic rate (i.e. percentage of completely metamorphosed larvae at different periods after settlement) of competent *T. gratilla* larvae.

MATERIALS AND METHODS

Laboratory assays. The effect of conditioned water treatments on the settlement and metamorphosis of *Tripneustes gratilla* larvae was determined in 2 experiments. A total of 10 full-sibling batches (different parentage) were cultured in the laboratory using standard protocols and feeding regime (Juinio-Meñez et al. 1998) at the University of the Philippines Marine Science Institute (UPMSI) hatchery at the Bolinao Marine Laboratory (BML). Only competent larvae (i.e. with well-developed rudiment) were used for the experiments. Boiled filtered (i.e. 5.0 to 10 µm) seawater (BFSW) was conditioned by placing fresh and ground *Sargassum* spp., adult *Salmacis sphaeroides* and adult *T. gratilla* in 4 separate 37 l aquaria with continuous aeration for 24 h. About 300 g of clean, whole *Sargassum* and finely chopped *Sargassum* were used to condition the water used for the fresh and the ground *Sargassum* treatments, respectively. Ten adult sea urchins (i.e. 6.0 to 7.0 cm test diameter) were used for each of the sea urchin conditioned treatments. In the second experiment, in lieu of ground *Sargassum*, 300 g of excreta collected from an aquarium with *Sargassum*-fed *T. gratilla* was used to simulate conditions of decomposing *Sargassum*. During the first experiment, a total of 6 different batches of competent full-sibling larvae belonging to 3 age groups (Group I: 35 to 37 d old; Group II: 49 d old; Group III: 56 to 57 d old) were tested. A series of 3 assays were conducted using 2 different full-sibling batches of larvae for each age group with 3 replicates for each treatment per batch of larvae. To determine differences among batches without the confounding effect of age, 4 different batches of larvae of the same age (37 d) were assayed simultaneously during the second experiment with 2 replicates

for each treatment per batch of larvae. The assays in the first experiment lasted for 24 h (i.e. when one treatment had at least 50% of larvae completely metamorphosed). To determine if the difference among treatments would change over a longer period, the assay period was extended to 96 h in the second experiment.

Settlement and metamorphosis were assayed in glass containers (90 mm diameter, 80 mm height) at room temperature (27 to 28°C) and ambient photoperiod (12 h light:12 h dark) and light intensity (0.74 to 57 $\mu\text{E m}^{-2} \text{s}^{-1}$). The total water volume in each container was 300 ml. For the conditioned water treatments, 150 ml of conditioned water was added to 150 ml of BFSW. Only BFSW was used for the control treatment. A film-free (i.e. cleaned with BFSW and UV-sterilized) plastic sheet (5.0 × 5.0 × 0.1 cm) was placed in each of the containers as a settlement substrate. Twenty competent larvae were placed in each assay container. In each container, the number of larvae that were swimming and attached on the substrate or glass with tube feet were determined after 1 h. The status of metamorphosis (i.e. completely metamorphosed, partially metamorphosed or not metamorphosed) was monitored under a stereoscope approximately every 4 h during the first day. In the second experiment, metamorphosis was monitored every 12 h starting Day 2. An individual was considered as completely metamorphosed when the pedicellariae appeared, the spines covered the entire test and it was actively moving or crawling; partially metamorphosed when the pedicellariae and very few spines were evident but the test was not yet completely round in shape, and it was starting to crawl; and not metamorphosed when settled but no observable changes seen except for the attached tube feet.

Statistical analysis. To determine differences in the mean percentage of settled larvae in each treatment after 1 h, a 1-way ANOVA on arc-sine transformed data was used, followed by Tukey's HSD test. To determine whether the rate of metamorphosis varied among treatments, differences in the mean percentage of completely metamorphosed individuals for selected monitoring periods were tested. Comparable data for the other observation periods were not obtained for all batches because scoring of the status of metamorphosis for all the batches of larvae could not always be completed within 1 to 2 h. For the first experiment (batches with different age groups), a 2-way ANOVA ($p < 0.05$) on arc-sine transformed percentages of completely metamorphosed *Tripneustes gratilla* was used to examine the effects of treatment, age and their interaction at the end of the experiment (24 h). For the second experiment (batches with the same age), a 2-way ANOVA ($p < 0.05$) on arc-sine transformed percentages of completely metamorphosed individuals was used to test the effect of treatment, batch and their

interaction after 24, 36, 72 and 96 h. Where significant effects were found, 1-way ANOVA ($p < 0.05$) on arc-sine transformed data, followed by Tukey's HSD test, was used to determine which means were significantly different from each other. Arcsine transformed data were subjected to a test of homogeneity of variances using Levene's tests prior to ANOVA. All statistical analyses were done with SPSS (SPSS 2001).

RESULTS

Induction of settlement

In both experiments and all treatments, more than 50% of all batches of larvae settled (i.e. not swimming in the water column) within 1 h of introduction to the assay containers, except for Batch 4 in the control treatment in the experiment with different age groups, where only 30% initially settled. There was no significant difference among the conditioned water treatments, but the percentage of settled larvae was significantly lower in the control for both experiments (Expt 1: $F = 10.1704$, $df = 4$, $p < 0.01$; Expt 2: $F = 11.2462$, $df = 4$, $p < 0.01$). After 1 h, the mean percentages of settled larvae in the conditioned water treatments ranged from 83.8 to 95.6%, while that in the control ranged from 65.3 and 73.1% in the 2 experiments.

Metamorphic rate

Larval batches of different age groups

The effects of treatment, age and their interaction on metamorphic rate were highly significant ($p < 0.01$; Table 1). Except in the fresh *Sargassum* treatment, the proportion of completely metamorphosed individuals

Table 1. Two-way ANOVA on the effects of 3 age groups and 5 treatments on the percentage of completely metamorphosed *Tripneustes gratilla* larvae after 4 and 24 h

Source	df	MS	F	p
After 4 h				
Treatment	4	0.05	6.62	<0.001
Age	2	0.15	17.9	<0.001
Treatment × Age	8	0.63	7.79	<0.001
Error	75	0.01		
After 24 h				
Treatment	4	1.47	57.84	<0.001
Age	2	1.41	55.78	<0.001
Treatment × Age	8	0.67	26.46	<0.001
Error	75	0.03		

increased over time. There were significant differences in the rates of metamorphosis among treatments. Completely metamorphosed juveniles were first observed in all conditioned water treatments after 4 h (Fig. 1). After 12 h, the mean percentages of completely metamorphosed individuals in the 2 *Sargassum*-conditioned treatments were significantly higher than the other treatments. However, after 24 h, it was highest in the ground *Sargassum* treatment and <1% of individuals metamorphosed completely in the control.

The mean percentage of larvae that were unmetamorphosed, partially metamorphosed and completely metamorphosed for the 6 batches in the different treatments in the first experiment are shown in Fig. 2. Compared to Batches 1 to 4, the percentages of completely metamorphosed larvae were lower in the youngest batches (5 and 6) for all treatments. For the oldest age group (Group III), the proportion of completely metamorphosed individuals was significantly higher in the *Sargassum* treatments starting the 4th hour until the end of the experiment (Table 2). Although not significantly different, after 4 h, the average proportion of completely metamorphosed larvae for the younger age group (Group I) was highest in the treatments conditioned with adult *Tripneustes gratilla*. In contrast, no larvae completely metamorphosed in the *Salmacis sphaeroides*-conditioned treatment. After 24 h, the percentage of completely metamorphosed individuals in the intermediate age group (Group II) was significantly higher in the adult sea urchin-conditioned treatments. Metamorphosis for this age group was also high

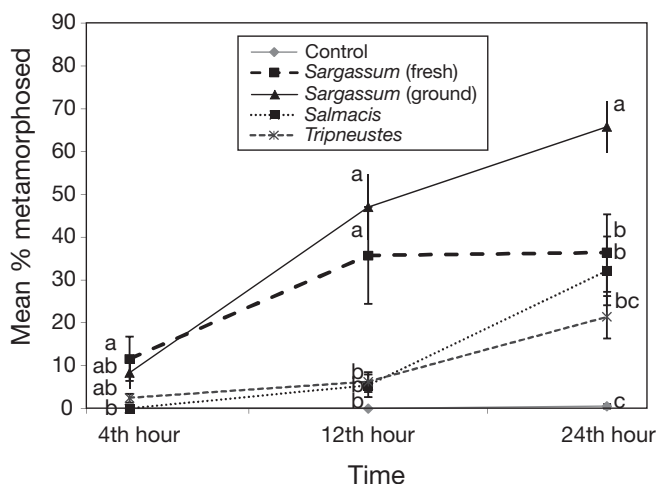


Fig. 1. *Tripneustes gratilla*. Mean percentage (\pm SE) of completely metamorphosed larvae after 4, 24 (n = 6 batches) and 12 h (n = 4 batches) in the 5 treatments in an experiment with different larval age groups. Two batches were not monitored during the 12th hour. Means with different letters vary significantly from each other (1-way ANOVA on arc-sine transformed data, Tukey's HSD test, $p < 0.05$)

in the ground *Sargassum* treatment and comparable to the oldest age group (Table 2). In the control treatment, the few completely metamorphosed larvae were found in the oldest age group.

Larval batches of the same age

In the second experiment, the age at induction (37 d) of all 4 larval batches was the same. The mean percentages of completely metamorphosed individuals after 24 h were much lower for all treatments (Fig. 3) than in the experiment with larval batches from different age groups (Fig. 1). The highest average percentage of completely metamorphosed larvae after 24 h was only 4.4%, in the fresh *Sargassum* treatment. Only after 72 h were >50% of larvae completely metamorphosed in the conditioned water treatments and a few completely metamorphosed in the control. The proportion of completely metamorphosed larvae increased in all treatments until the end of the experiment (96 h), ranging from an average of 69.4 to 79.4% in the conditioned water treatments compared to only 35% in the control. There was no significant difference in the proportion of completely metamorphosed larvae among conditioned water treatments, but these treatments were significantly higher than the control.

Two-way ANOVA of the mean percentages of completely metamorphosed larvae among 4 larval batches showed significant differences with respect to treatments and batches during different observation periods. Moreover, there was a significant interaction between treatment and batch after 72 h (Table 3). Notably, Batches 7 and 8 metamorphosed earlier (24 and 36 h) in the sea urchin-conditioned treatments (Table 4). By the end of the second experiment (96 h), there were significant differences among batches only in the *Tripneustes gratilla*-conditioned treatment.

DISCUSSION

Effects of habitat-associated cues

Larvae of different benthic marine invertebrates have different degrees of specificity with respect to natural inducers of settlement and metamorphosis (Morse 1990, Bishop et al. 2006). Bacterial films or biofilms enriched with diatoms have been shown to induce settlement and metamorphosis in competent larvae of several echinoderm species (reviewed in Hadfield & Paul 2001). The sea urchins *Strongylocentrotus droebachiensis* (Pearce & Scheibling 1991), *Paracentrotus lividus* (Gosselin & Jangoux 1996) and *Tripneustes gratilla* (Dworjanyn & Pirozzi 2008) have

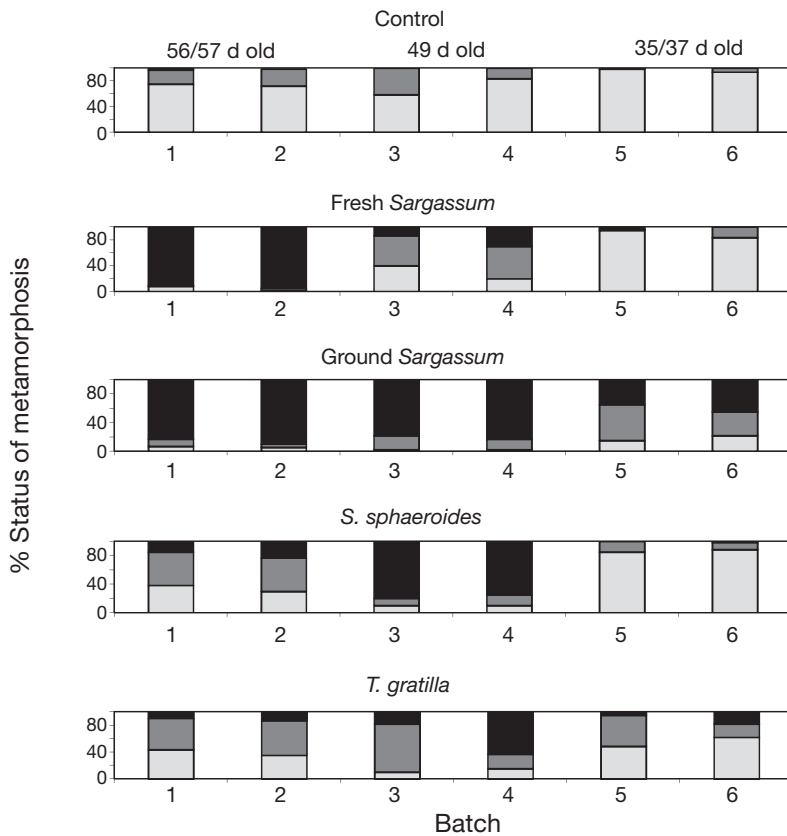


Fig. 2. *Tripneustes gratilla*. Mean percentage of larvae at different stages of metamorphosis in 6 batches (n = 3 replicates per batch) and 3 age groups 24 h after settlement. Light grey: unmetamorphosed; mid-grey: partially metamorphosed; black: completely metamorphosed

been shown to settle on a variety of macroalgae and biofilms, which indicates low substratum specificity.

In addition, conspecific-associated cues also induced settlement in *Tripneustes gratilla* but not in other sea urchin species (Dworjanyan & Pirozzi 2008). Sand dollars that occur in dense aggregations have been shown to settle and metamorphose in response to conspecifics (Pearce & Scheibling 1990b). In contrast, larvae of

Strongylocentrotus intermedius metamorphosed only in old seagrass colonized with a specific epiphytic calcareous alga (Naidenko 1996). In the present study we investigated the effects of waterborne cues from conspecific and sympatric adult sea urchins and different forms of *Sargassum*, a common natural food of *T. gratilla*, to gain insights on the importance of these extrinsic factors in relation to intrinsic factors, specifically age and parentage, on larval settlement and metamorphic success in this species. Results of both experiments showed that waterborne cues in the conditioned water treatments enhanced settlement. The majority of the larvae ranging from 35 to 57 d old settled immediately upon introduction in water conditioned with sea urchins (i.e. conspecific and sympatric adults) and *Sargassum* (i.e. fresh, ground and feces). Interestingly, although significantly lower than the conditioned water treatments, 65 and 73% of larvae across age groups also settled in the control (i.e. BFSW) treatment in 2 experiments, respectively. Moreover, in the experiment with similarly aged larval batches, over 30% of the larvae were able to completely metamorphosis after 96 h in the control

treatment. These results clearly indicate that competent *T. gratilla* larvae have very low cue specificity for settlement induction. Dworjanyan & Pirozzi (2008) found that a wide range of cues induced settlement in *T. gratilla* larvae (35 d old). However, in contrast to our findings, they found no settlement in the control treatments (UV-sterilized seawater) even after 48 h, and suggested that cues are obligatory to induce settle-

Table 2. *Tripneustes gratilla*. Mean (\pm SE) percentage of completely metamorphosed larvae by age group after 4 and 24 h in the 5 treatments. Age group means per treatment with different superscripted letters vary significantly from each other (1-way ANOVA on arc-sine transformed data, Tukey's HSD test, $p < 0.05$). F: fresh; G: ground

Age group	Larval batch	Mean % metamorphosed				
		Control	<i>Sargassum</i> (F)	<i>Sargassum</i> (G)	<i>Tripneustes</i>	<i>Salmacis</i>
After 4 h						
I (35–37 d)	5 & 6	0	0.00 ^b	0.83 ^b \pm 0.83	8.33 ^a \pm 3.12	0
II (49 d)	3 & 4	0	0.00 ^b	0.00 ^b	1.67 ^a \pm 1.05	0
III (56–57 d)	1 & 2	0	35.00 ^a \pm 10.65	24.17 ^a \pm 7.24	1.67 ^a \pm 1.05	0
After 24 h						
I (35–37 d)	5 & 6	0	1.67 ^b \pm 1.05	40.00 ^b \pm 4.65	18.33 ^b \pm 4.71	0.83 ^a \pm 0.83
II (49 d)	3 & 4	0	21.67 ^b \pm 4.22	70.83 ^a \pm 10.28	40.83 ^a \pm 10.28	76.67 ^a \pm 2.79
III (56–57 d)	1 & 2	1.67 ^a \pm 1.67	85.83 ^a \pm 6.38	86.67 ^a \pm 3.33	11.67 ^b \pm 3.07	19.17 ^b \pm 4.17

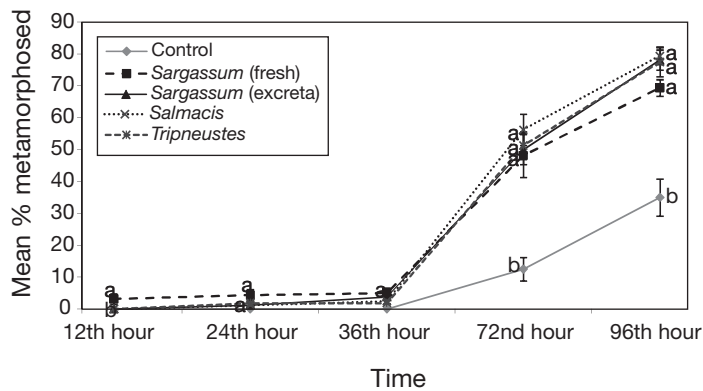


Fig. 3. *Tripneustes gratilla*. Mean percentage (\pm SE) of completely metamorphosed larvae ($n = 4$ batches) at different monitoring periods in the 5 treatments in an experiment with larval batches of the same age. Means with different letters vary significantly from each other (1-way ANOVA on arcsine transformed data, Tukey's HSD test, $p < 0.05$)

Table 3. Two-way ANOVA on the effects of 4 larval batches of the same age and 5 treatments on the percentage of completely metamorphosed *Tripneustes gratilla* larvae at 24, 36, 72 and 96 h

Source	df	MS	F	p
24 h				
Treatment	4	0	2.79	0.054
Batch	3	0	3.88	0.024
Treatment \times Batch	12	0	1.18	0.36
Error	20	0		
36 h				
Treatment	4	0	3.06	0.04
Batch	3	0.01	7.53	0.001
Treatment \times Batch	12	0	1.65	0.157
Error	20	0		
72 h				
Treatment	4	0.28	20.12	<0.001
Batch	3	0.07	5.1	0.009
Treatment \times Batch	12	0.06	4.51	0.002
Error	20	0.01		
96 h				
Treatment	4	0.046	41.91	<0.001
Batch	3	0.09	7.88	0.001
Treatment \times Batch	12	0.03	30.59	0.013
Error	20	0.01		

ment. The use of only competent larvae in the assays in the present study may account for the differences in the settlement induction results. Larval batches in the youngest age group (35 to 37 d old) in our experiments also settled in the control treatment.

After settlement, the percentage of completely metamorphosed larvae in the conditioned water treatments was significantly higher compared to the control (BFSW) during all observation periods in both experi-

ments. However, the rate of metamorphosis in the different conditioned water treatments varied significantly. In the experiment with the different age groups, completely metamorphosed larvae were observed earlier in the *Sargassum*-conditioned treatments. After 24 h, the percentage of early juveniles was significantly higher in the ground *Sargassum* treatment compared with the other treatments, including the fresh *Sargassum* treatment. *Sargassum*-conditioned water enhanced the rate of metamorphosis particularly in older larvae. Likewise in the experiment with the larval batches of the same age, more batches had completely metamorphosed juveniles during the first 36 h in the *Sargassum*-conditioned treatments. Dworjanyn & Pirozzi (2008) found both algal substrate and water conditioned with *S. linearifolium* induced high settlement and initial metamorphosis in *Tripneustes gratilla*. However, the rate of metamorphosis or percentage of larvae that completely metamorphosed into juveniles was not determined. They also reported that cleaning of the algal surface to reduce bacterial abundance significantly reduced larval settlement, indicating that larvae settled due to cues from associated bacteria rather than the algae per se. Lower settlement of larvae in cleaned surfaces of coralline algae with reduced bacterial community was likewise reported in the common Australian sea urchin *Heliocidaris erythrogramma* (Huggett et al. 2006). However, the incidence of aggregations of early juvenile *Tripneustes gratilla* on *Sargassum* spp. in the reef flats of Bolinao and Ilocos, northwestern Philippines, especially during the peak season of the *Sargassum* spp. and not on other macroalgae (H. G. P. Bangi pers. obs.), indicate that specific algal metabolites may also be important. In addition, ground *Sargassum* extract has been routinely used with benthic diatoms to induce larval settlement and as supplemental feed for early juveniles at the BML hatchery since 1996. Other studies have shown that compounds isolated from algae such as histamine (Swanson et al. 2004, 2007), polyunsaturated fatty acids (Kitamura et al. 1992, 1993) and glycolipids (Takahashi et al. 2002) induce settlement and metamorphosis in sea urchin larvae.

Dworjanyn & Pirozzi (2008) found that the presence of juvenile (5 and 10 mm test diameter) *Tripneustes gratilla* and their feces induced significantly higher settlement of *T. gratilla* larvae compared to water and surfaces conditioned with juveniles and controls. In the present study, water conditioned with conspecific adults and excreta of *T. gratilla* that were fed with *Sargassum* did not have significantly higher percentages of completely metamorphosed juveniles compared to the other conditioned water treatments. Water conditioned with adult conspecifics as well as a sympatric species, *Salmacis spaeroides*, induced set-

Table 4. *Tripneustes gratilla*. Mean (\pm SE) percentage of completely metamorphosed larvae (4 batches) of the same age after 24, 36, 72 and 96 h in the 5 treatments. Batch means with different superscripted letters vary significantly from each other (1-way ANOVA on arc-sine transformed data, Tukey's HSD test, $p < 0.05$). F: fresh; E: excreta

Larval batch	Mean% metamorphosed				
	Control	<i>Sargassum</i> (F)	<i>Sargassum</i> (E)	<i>Tripneustes</i>	<i>Salmacis</i>
After 24 h					
7	0	7.50 ^a \pm 2.50	0.00 ^a	0.00 ^b	2.50 ^a \pm 2.50
8	0	5.00 ^a \pm 5.00	5.00 ^a \pm 0.00	7.50 ^a \pm 2.50	2.50 ^a \pm 2.50
9	0	5.00 ^a \pm 5.00	0.00 ^a	0.00 ^b	0.00 ^a
10	0	0.00 ^a	0.00 ^a	0.00 ^b	0.00 ^a
After 36 h					
7	0	7.50 ^a \pm 2.50	0.00 ^b	0.00 ^b	2.50 ^a \pm 2.50
8	0	5.00 ^a \pm 5.00	12.50 ^a \pm 2.50	7.50 ^a \pm 2.50	7.50 ^a \pm 2.50
9	0	5.00 ^a \pm 5.00	2.50 ^{ab} \pm 2.50	0.00 ^b	0.00 ^a
10	0	2.50 ^a \pm 2.50	0.00 ^b	0.00 ^b	0.00 ^a
After 72 h					
7	7.50 ^a \pm 7.50	27.50 ^a \pm 7.50	32.50 ^a \pm 2.50	32.50 ^b \pm 2.50	65.00 ^a \pm 10.00
8	27.50 ^a \pm 2.50	37.50 ^a \pm 2.50	60.00 ^a \pm 10.00	55.00 ^a \pm 5.00	42.50 ^a \pm 7.50
9	7.50 ^a \pm 2.50	62.50 ^a \pm 2.50	47.50 ^a \pm 2.50	57.50 ^a \pm 2.50	60.00 ^a \pm 15.00
10	7.50 ^a \pm 2.50	65.00 ^a \pm 15.00	60.00 ^a \pm 0.00	60.00 ^a \pm 0.00	57.50 ^a \pm 2.50
After 96 h					
7	20.00 ^a \pm 10.00	65.00 ^a \pm 5.00	65.00 ^a \pm 5.00	60.00 ^b \pm 5.00	80.00 ^a \pm 0.00
8	57.50 ^a \pm 2.50	62.50 ^a \pm 2.50	82.50 ^a \pm 2.50	90.00 ^a \pm 5.00	72.50 ^a \pm 2.50
9	30.00 ^a \pm 5.00	75.00 ^a \pm 0.00	82.50 ^a \pm 2.50	85.00 ^{ab} \pm 5.00	80.00 ^a \pm 10.00
10	32.50 ^a \pm 7.50	75.00 ^a \pm 5.00	82.50 ^a \pm 2.50		

tlement and complete metamorphosis of larvae. Given our observations on aggregations of early recruits in *Sargassum* beds, gregarious settlement of this species may be linked to algal habitat. In the hatchery, early juveniles feed on mixed benthic diatoms and start feeding on ground *Sargassum* upon reaching a size of >5 mm test diameter. Aside from protection, *Sargassum* fronds harbor a wide variety of benthic diatoms that newly settled *T. gratilla* may feed on. In 7 locations in the western Sargasso Sea, the benthic diatom *Navicula* sp. and 12 other species of diatoms were reported to be attached to *Sargassum* (Carpenter 1970). In addition, the higher metamorphic rate of *T. gratilla* in the ground *Sargassum* treatment which would have more algal exudates compared to the fresh *Sargassum* treatment, which further suggests that algal metabolites and their associated diatoms and bacteria may enhance benthic recruitment in *T. gratilla* in nature.

Effects of age and parentage

Metamorphic rate was significantly affected by age. The lowest percentages of completely metamorphosed individuals were found in the youngest age group (35 to 37 d old) and completely metamorphosed larvae in the control treatment were found in only one of the older batches (Table 2, Fig. 2). The overall slower rate of metamorphosis in the second experiment may be

attributed to the younger age of larvae (37 d old) during induction. Likewise, the increase in the incidence of 'spontaneous metamorphosis' (Bishop et al. 2006) in the control treatment over time shows that larvae attain greater metamorphic competence with age. Change in metamorphic competence with age has been reported in some marine invertebrates (e.g. Jackson et al. 2005, Gribben et al. 2006). In the Australian sea urchin *Holopneustes purpurascens*, older larvae maintained a stringent requirement for exposure to histamine in order to metamorphose, but metamorphosed in response to lower concentrations of histamine in brown algae, which contain much lower levels of histamine than red algae (Swanson et al. 2007). It was suggested that older larvae might expand their range of settlement preferences with age by changing their sensitivity to a single settlement substratum. In contrast, *Tripneustes gratilla* has planktotrophic larvae with low cue specificity for settlement and metamorphosis. Since the feeding history of all batches of larvae prior to induction was similar across treatments, the observed differences in metamorphic rate and success among different age groups may reflect differences in the ability of larvae to detect cues that can initiate and complete metamorphosis rather than energy limitation, as suggested in the DLH and the model to compare conditions under which decreasing selectivity should be favoured (Elkin & Marshall 2007). Interestingly, there was a significant interaction between age and treatment in the first experiment (Table 1). The

response of younger larvae, particularly the intermediate age group, to water conditioned with adult sea urchins was higher compared to the oldest age group, which had higher metamorphic rate in the *Sargassum* treatments (Table 2). These results further suggest that chemoreceptors necessary for induction and completion of metamorphosis in *T. gratilla* may initially be more sensitive to cues from other sea urchins and/or that there is polymorphism in the sensitivity of larvae to metamorphic cues.

The use of full-sibling larval batches of the same age allowed us to examine the effect of genetic factors independent of larval age. Significant differences in the percentages of completely metamorphosed individuals among batches and significant interaction between batch and treatment (Table 3) indicate familial or genetic differences in terms of sensitivity to different types of habitat-associated cues. Botello & Krug (2006) also found significant between-family differences, suggesting that response of the larvae of the gastropod *Alderia* sp. to a low dose of the dissolved settlement cue from the alga *Vaucheria longicaulis* may have a heritable component. Results of the present study lend support to the proposal of Jackson et al. (2005) that specific receptors and/or components of intracellular pathways may in part be genetically determined and that loci regulating the acquisition of competence are polymorphic.

The high incidence of 'spontaneous metamorphosis' in the control treatment of the present study showed that competent *Tripneustes gratilla* can settle and metamorphose without habitat-associated waterborne cues. However, different water conditioned with sea urchins and various forms of *Sargassum* enhanced metamorphic rates and yielded significantly more completely metamorphosed early juveniles. *Sargassum*-associated cues enhanced metamorphosis, particularly in older larvae, while intermediate aged larvae had a higher response to water conditioned with adult sea urchins (i.e. conspecifics and a sympatric species). Aside from habitat-associated cues, the age of competent larvae and genetic factors may affect intensity of settlement and early benthic recruitment patterns in nature, and is also important to consider in the cultivation of this species.

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