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ORIGINAL PAPER

Dissolved histamine: a potential habitat marker promoting settlement and metamorphosis in sea urchin larvae

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Abstract Many species of marine invertebrate larvae settle and metamorphose in response to chemicals produced by organisms associated with the adult habitat, and histamine is a cue for larvae of the sea urchin *Holopneustes purpurascens*. This study investigated the effect of histamine on larval metamorphosis of six sea urchin species. Histamine induced metamorphosis in larvae of three lecithotrophic species (*H. purpurascens*, *Holopneustes inflatus* and *Heliocidaris erythrogramma*) and in one planktotrophic species (*Centrostephanus rodgersii*). Direct comparisons of metamorphic rates of lecithotrophic and planktotrophic larvae in assays cannot be made due to different proportions of larvae being competent. Histamine (10 μM) induced metamorphosis in 95% of larvae of *H. purpurascens* and *H. inflatus* after 1 h, while the coralline alga *Amphiroa anceps* induced metamorphosis in 40–50% of these larvae. Histamine (10 μM) and *A. anceps* induced 40 and 80% metamorphosis, respectively, in the larvae of *H. erythrogramma* after 24 h. Histamine (10 μM) and the coralline alga *Corallina* sp. induced 30 and 70% metamorphosis, respectively, in the larvae of *C. rodgersii* after 24 h. No metamorphosis of any larval species occurred in seawater controls. Larvae of two planktotrophic species (*Tripneustes gratilla* and *Heliocidaris tuberculata*) did not metamorphose in response to histamine. Seagrasses, the host plants of *H. inflatus*, induced rapid metamorphosis in larvae of the two *Holopneustes* species, and several algae induced metamorphosis in *C. rodgersii* larvae. Histamine leaching from algae and seagrasses may act as a habitat marker and metamorphic cue for larvae of several ecologically important sea urchin species.

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Introduction

Marine invertebrate larvae may spend days, weeks or months in the plankton, depending on their mode of development. Larval settlement, metamorphosis and recruitment into adult populations are fundamental ecological processes that help determine the structure of benthic communities (Underwood and Keough 2000). A number of studies have shown that marine larvae are not passive participants during the critical life-history transition marked by settlement out of the plankton and metamorphosis (Butman 1987; Finelli and Wethey 2003). The larvae of echinoderms, molluscs and polychaetes respond to environmental factors that influence the timing and/or site of settlement and metamorphosis (Crisp 1974; Pawlik 1992). Most larvae become competent to settle and metamorphose after a period of development in the plankton (Strathmann 1985). The settlement process often begins with the descent of competent larvae to the benthos, which is followed by benthic exploration and sometimes attachment. Metamorphosis is defined

by the loss of larval features and the irreversible morphological transformation into the juvenile (Hadfield 2000). Even though settlement and metamorphosis are separate phenomena, they generally occur in close succession in response to an inducer referred to as a metamorphic cue (reviewed by Hadfield and Paul 2001; Hadfield 2010). Metamorphic cues may act as signposts for marine invertebrate larvae indicating favourable habitats for future success (Larsson and Jonsson 2006). A number of strategies are utilised by competent larvae for settlement and metamorphosis (Pechenik 1990; Bishop et al. 2006). Some larvae have stringent requirements for metamorphosis, particularly larvae of habitat/feeding specialists such as the corallivorous nudibranch *Phestilla sibogae* (reviewed by Hadfield 1998) and the opisthobranch *Alderia modesta* (Krug and Manzi 1999). In other species, spontaneous metamorphosis of larvae occurs in the absence of cues at the onset of competency or in older larvae (Fenaux and Pedrotti 1988; Gibson and Chia 1995; Krug 2001).

Metamorphic cues can be physical factors such as light (Baird et al. 2003) and surface texture (Berntsson et al. 2004), or more complex biological and chemical cues (Keough and Raimondi 1995; Clare and Matsumura 2000). The most commonly reported metamorphic cues are chemicals produced by organisms associated with the juvenile/adult habitat such as prey (Hadfield and Scheuer 1985), hosts (Williamson et al. 2000), conspecifics (Burke 1984) or microbial biofilms (Weiczorek and Todd 1998). Chemical cues for metamorphosis range from large, insoluble, surface-bound glycoproteins and carbohydrates to small peptides dissolved in seawater (Zimmer-Faust and Tamburri 1994; Krug and Manzi 1999; Clare and Matsumura 2000). Although the notion that chemicals dissolved in seawater could affect the behaviour of settling larvae was initially dismissed (Crisp 1974), many species are now known to respond to waterborne cues (Hadfield and Scheuer 1985; Turner et al. 1994; Krug and Manzi 1999; Finelli and Wethey 2003). Numbers of naturally occurring, surface-bound and dissolved cues inducing metamorphosis in invertebrate larvae have been partially described (e.g., proteinaceous), but only a few have been completely characterised (Yvin and Chevolet 1985; Tsukamoto et al. 1999; Swanson et al. 2004).

Histamine is a naturally occurring chemical dissolved in seawater that induces metamorphosis in the lecithotrophic larvae of the Australian sea urchin *Holopneustes purpurascens* (Swanson et al. 2004). *Holopneustes purpurascens* is an unusual urchin because it lives in the canopy of its host plants, *Delisea pulchra* and *Ecklonia radiata*, consuming the algae that it also uses as habitat (Steinberg 1995). Larvae of *H. purpurascens* metamorphose rapidly in the presence of the red alga *D. pulchra,* in response to a metamorphic cue derived from this alga that is dissolved in seawater (Williamson et al. 2000). This cue was subsequently identified as histamine (Swanson et al. 2004). Histamine at 10 μM triggers rapid metamorphosis in larvae of *H. purpurascens* in static bioassays (Swanson et al. 2004, 2007). Histamine is produced in high quantities by *D. pulchra* (~100–300 μg g^{-1} dw [dry weight]) and in lower quantities by the kelp *E. radiata* and brown algae *Sargassum linearifolium* and *Homeostrichus olsenii* (0.1–3.0 μg g^{-1} dw; Swanson et al. 2006). This chemical is also present in trace amounts concentrated on the surface of coralline algae (0.01–0.1 μg g^{-1} dw, Tebben 2008), which are known to provide metamorphic cues for the larvae of corals, molluscs and echinoderms (reviewed by Hadfield and Paul 2001). Red (including corallines) and brown algae induced metamorphosis in larvae of *H. purpurascens* in the laboratory with varying efficacy (Williamson et al. 2000; Swanson et al. 2006; Tebben 2008). Very low concentrations of histamine (~5–15 nM) were measured in seawater collected adjacent to *D. pulchra* (Swanson et al. 2006; Tebben 2008), and some of these samples induced metamorphosis of larvae of *H. purpurascens* (Swanson et al. 2006). These findings are the only instance where a characterised metamorphic cue has been quantified in the habitat and shown to relate to the recruitment of the organism (Swanson et al. 2006).

The discovery that histamine acts as a metamorphic cue for larvae of *H. purpurascens* is intriguing as this is a well-known neural signalling molecule in invertebrates and vertebrates (Reite 1972; Stuart 1999). This study addressed the hypothesis that histamine is a common metamorphic cue for sea urchin larvae. Sea urchins exhibit a diverse array of developmental modes ranging from simple non-feeding (lecithotrophic) larvae that develop in several days, to feeding (planktotrophic) larvae that develop over weeks or months (Strathmann 1985). Here, we investigated whether histamine acts as a metamorphic cue for the larvae of six sea urchins, three with lecithotrophic development (*H. purpurascens*, *Holopneustes inflatus* and *Heliocidaris erythrogramma*) and three with planktotrophic development (*Heliocidaris tuberculata, Tripneustes gratilla* and *Centrostephanus rodgersii*), species for which development and metamorphosis are well characterised (Morris 1995; Byrne et al. 2001; Huggett et al. 2006; Dworjanyn and Pirozzi 2008; Soars et al. 2009). These species are also common and ecologically important in intertidal and sublittoral habitats. Four of these species co-occur, and so their larvae might use similar cues for settlement and metamorphosis.

Methods

Gamete collection and larval culture

Six echinoid species representing three orders (Table 1) were collected from the shallow sublittoral at several sites

Table 1 Taxonomy of sea urchins in the study and their mode of larval development (*L* lecithotrophic, *P* planktotrophic), type of larva produced and age of larvae used in assays

Order	Family	Species	Larval development		
			Mode	Type	Age
Temnopleuroida	Temnopluridae	*Holopneustes purpurascens*	L	Ovoid	6 days
		Holopneustes inflatus	L	Ovoid	6 days
	Toxopneustidae	*Tripneustes gratilla*	P	Echinopluteus	5 weeks
Echinoida	Echinometridae	*Heliocidaris erythrogramma*	L	Reduced pluteus	4 days
		Heliocidaris tuberculata	P	Echinopluteus	7 weeks
Diadematacea	Diadematidae	*Centrostephanus rodgersii*	P	Echinopluteus	11 weeks

Table 2 Experimental conditions of each assay: concentrations of histamine tested, replication, alga used to measure competency and times at which the assay was scored (*tmt* treatment, *A Amphiroa anceps*, *C Corallina officinalis*)

Species	Concentrations (μM)	# dishes/tmt (# larvae/dish)	Alga	Score (h)
Holopneustes purpurascens	10, 1, 0.1 and 0.01	10 (10)	A	1, 24, 48
H. inflatus	10, 1, 0.1 and 0.01	10 (10)	A	1, 24, 48
Heliocidaris erythrogramma	200, 100, 50, 10 and 1	10 (5)	A	1, 24
H. tuberculata	200, 100, 50, 10 and 1	5 (5)	C	24, 48
Tripneustes gratilla	100, 10, 1 and 0.1	10 (10–20)	C	48
Centrostephanus rodgersii	100, 10 and 1	10 (20–30)	C	24, 48

along the coast of New South Wales, Australia, between September 2003 and July 2011. The following urchins were collected from Botany Bay (Sydney) during their reproductive periods: *Holopneustes* spp. (spring 2004, Williamson and Steinberg 2002), *Heliocidaris erythrogramma* and *Heliocidaris tuberculata* (spring 2003 and winter 2004, respectively, Laegdsgaard et al. 1991). The Coffs Harbour populations of *Tripneustes gratilla* and *Centrostephanus rodgersii* are reproductive in summer and winter, respectively; however, broodstock held at the National Marine Science Centre (NMSC—Coffs Harbour) are reproductive year round (Byrne et al. 1998; Mos et al. 2011). Broodstock of *T. gratilla* and *C. rodgersii* held at the NMSC were spawned in February 2005 and July 2011, respectively. Urchins (except *Holopneustes* spp.) were spawned by intracoelomic injection of 2–5 mL of 0.5–2.0 M KCl. For each of these species, embryos were pooled from two separate fertilisations of at least 3 females and 3 males. For *H. purpurascens* and *H. inflatus,* 20 urchins of each species were held in separate 20-L buckets with air until they spawned pooling the embryos from each bucket (usually within ~48 h of collection).

Seawater was either autoclaved and antibiotics added (22 mg L^{-1} of penicillin G and 37 mg L^{-1} of streptomycin sulphate–sterile seawater, SSW) or was filtered (1-μm) and UV-sterilised (FSW). Larvae were cultured at the ambient temperature at which adults were collected. The lecithotrophic larvae (*H. purpurascens*, *H. inflatus* and *H. erythrogramma)* were cultured at 19°C in SSW (salinity, 36) in 2-L beakers with gentle aeration. Lecithotrophic larvae were stocked at a maximum density of 4-larvae mL^{-1}. Seawater (SSW) was changed daily until competency (3–5 days). The echinoplutei of *H. tuberculata* were cultured at 19°C in FSW (salinity, 36) in 2-L beakers and stirred gently with a paddle system. Larvae were stocked at an initial density of 10 larvae mL^{-1}, which decreased to 2 larvae mL^{-1} after 1 week and 1 larva mL^{-1} by 7 week. These larvae were fed *Chaetoceras muelleri* at 2×10^4 cells mL^{-1} every other day from 3rd day onwards, and FSW was changed every 3–4 days until competency (~7 week). *Tripneustes gratilla* and *C. rodgersii* were cultured at 25 and 21°C, respectively, in FSW (salinity, 37) in 125-L rearing tanks. Larvae were initially stocked at 5 larvae mL^{-1}, which was reduced to 1 larva mL^{-1} towards the end of the rearing period. These larvae were fed *Proteomonas sulcata* at 5×10^3 cells mL^{-1} daily from 3rd day onwards and gradually increased to 4×10^4 cells mL^{-1} at the 8-arm pluteus stage (~5 and 11 weeks, respectively). Seawater in which larvae of *T. gratilla* and *C. rodgersii* were cultured was cleaned daily by exchanging two tank volumes of FSW, and larval cultures were transferred into clean tanks weekly.

Histamine assays

All assays followed the same basic protocol; specific details for each assay are shown in Table 2. The response of larvae of *H. purpurascens* to histamine is well established (Swanson et al. 2004, 2006, 2007). The effects of histamine on larvae of *H. purpurascens* and *H. inflatus* were tested to directly compare the response of two closely related species. All tests were done in static conditions under a 12-h light : 12-h dark photoperiod at the temperature at which larvae were cultured (19–25°C). Replicates were randomly assigned among treatments. Diluted stock solutions of

histamine in seawater were prepared on the day of the assay from a concentrated stock solution of histamine (10 mg mL^{-1} in Milli-Q). Aliquots of diluted stock solutions of histamine were added to sterile Petri dishes (36 mm) followed by 4–5 mL of SSW or FSW. Histamine concentrations tested ranged from 0.01 to 200 μM with a subset of concentrations tested on each species depending on the availability of larvae. The higher concentrations of histamine (>10 μM) were not tested against larval *Holopneustes* spp. as a maximal response was observed with 10-μM histamine.

The rate of development of larvae to competency can vary within a single cohort, particularly for planktotrophic species that develop over a number of weeks (Hadfield and Strathmann 1996). Thus, a proportion of larvae used in each assay may not have been competent to metamorphose. Coralline algae known to induce metamorphosis of larvae of *Holopneustes* spp. (Swanson et al. 2006), *H. erythrogramma* (Huggett et al. 2006), *H. tuberculata* (Byrne et al. 2011), *T. gratilla* (Dworjanyn and Pirozzi 2008) and *C. rodgersii* (herein) were used in each assay to indicate the proportion of larvae that were competent to metamorphose (Table 2). Lecithotrophic larvae used in assays were 6 days old for *Holopneustes* species and 4 days old for *H. erythrogramma,* that is, one day older than the typical age that these larvae attain competence in our hands (Table 1). Larvae of *H. tuberculata* were deemed to be competent at 5 weeks old when, in a preliminary assay, >90% of a subset of larvae metamorphosed in the presence of the coralline alga *Corallina officinalis*. Larvae of *T. gratilla* were used in assays at 5 weeks old when at least 50% had large rudiments and were deemed competent to metamorphose (Dworjanyn and Pirozzi 2008). Larvae of *C. rodgersii* were 11 weeks old at the time of the assay and had multiple pedicellaria and/or tube-feet, structures indicating competency (B. Mos and S. Dworjanyn, in prep.). Sterile seawater or FSW treatments were used as controls for spontaneous metamorphosis. Larvae were added once all dishes were prepared. Planktotrophic larvae were not fed during the histamine assay. The number of larvae used for each species depended on their availability. Percent metamorphosis was recorded over time (h) in repeated observations (Table 2). Attachment, an early stage of the settlement process, was scored in tests with *H. tuberculata* as a significant proportion of larvae showed this behaviour, but did not metamorphose, in response to histamine. A larva was deemed to have attached firmly to the dish if it could not be dislodged by a gentle stream of water from a pipette.

Algal and seagrass assays

Investigations into the effects of algae on metamorphosis of larval *H. purpurascens*, *H. erythrogramma* and larval *T. gratilla* have been reported elsewhere (Huggett et al. 2006; Swanson et al. 2006; Dworjanyn and Pirozzi 2008). Following the induction of metamorphosis of larval *H. inflatus* and *C. rodgersii* by histamine, seagrasses and algae from the habitat were tested in assays with larvae to see whether they induced metamorphosis. There were not enough *H. tuberculata larvae* available to do an algal assay.

Host plant assay with *Holopneustes* species

Seagrasses, the host plants for *H. inflatus*, were assessed to see whether they would induce metamorphosis in larvae of *H. inflatus*. As the response of larval *H. inflatus* and *H. purpurascens* to histamine was similar, the effects of common host plants of both species were investigated in this assay with both larval species. Three species of seagrass (*Posidonia australis, Halophila ovalis* and *Zostera capricornia)*, the algae *Delisea pulchra* and *Amphiroa anceps,* and the kelp *Ecklonia radiata* were collected on the day of the assay. Six-day-old larvae of *H. inflatus* ($N = 10$ [3 per dish]) and *H. purpurascens* ($N = 10$ [5 per dish]) were added to dishes containing 4 mL of SSW and 10–30 mg of seagrass or alga, or histamine (10 μM). Percent metamorphosis was scored at 1 and 24 h.

Algal assay with *Centrostephanus rodgersii*

Algae common in the habitat of *Centrostephanus rodgersii* were assessed for metamorphic activity. The following algae were collected on the day of the assay: the red algae *A. anceps*, *Corallina officinalis* and *Laurencia* sp.; the brown algae *Sargassum linearifolium, Dictyota dichotoma, Dilophus marginata* and *Ecklonia radiata*; and the green alga *Ulva* sp. Eleven-week-old larvae of *C. rodgersii* ($N = 10$ [20–30 per dish]) were added to dishes containing 4 mL of FSW and 15 mm^2 of algae. Percent metamorphosis was scored at 24 and 48 h.

Statistical analysis

Proportional data were transformed (arcsine) prior to analysis. Percent metamorphosis of larvae of *H. purpurascens* and *H. inflatus* in response to histamine were analysed by repeated measures 2-factor ANOVA (with species and histamine concentration as random factors). Percent metamorphosis of larvae *H. purpurascens* and *H. inflatus* to seagrass and algae was analysed by a repeated measures 2-factor ANOVA (with species and plant as fixed factors). All other analyses (percent metamorphosis of larvae of *H. erythrogramma* in response to histamine at 24 h, percent attachment of larvae of *H. tuberculata* in response to histamine at 48 h and percent metamorphosis of *C. rodgersii* in response to histamine and algae at 24 h) were conducted by permutational multivariate analysis of variance (PERMANOVA)

using Primer 6 (Primer-E, Plymouth) with PERMANOVA$^+$ extension (v.6.1.7) software. PERMANOVA was used here as proportional data did not meet the assumptions of normality and/or constant variance required for ANOVA (Anderson 2001; McArdle and Anderson 2001; Anderson 2005). Pair-wise comparisons of untransformed data were generated using Euclidean distance, utilising approximately 9,999 permutations of the raw data. Monte Carlo *P*-values (P-$_{MC}$) were used when the number of unique permutations was low (Anderson 2005). Pair-wise post hoc tests were performed if PERMANOVA results indicated that there were significant differences between treatments.

Results

Histamine assays

Species with lecithotrophic larvae

Histamine (10 µM) induced rapid metamorphosis in >90% of larvae of *Holopneustes purpurascens* and *H. inflatus* within 1 h of exposure (Fig. 1). Histamine (1 µM) induced metamorphosis in 35–40% of larvae of both species of *Holopneustes* after 1 h, which increased to 90–95% metamorphosis by 24 h (Fig. 1). The geniculate coralline alga *Amphiroa anceps* induced metamorphosis in 45–50% of larval *H. purpurascens* and *H. inflatus* after 1 h, which increased to 95% of larvae by 24 h (Fig. 1). Lower concentrations of histamine (0.01 and 0.1 µM) and SSW induced minimal metamorphosis (<5%) in both species after 24 h. Thus, histamine induced metamorphosis in larvae of *H. inflatus* at the same level as for larvae of *H. purpurascens,* at the range of concentrations tested (Fig. 1, Table 3; species effect: $F_{(1,90)} = 0.662$, $P = 0.418$).

There was no effect of histamine or *A. anceps* on larvae of *Heliocidaris erythrogramma* after 1 h. By 24 h, however, histamine (10 µM) induced metamorphosis in 40% of *H. erythrogramma* larvae while *A. anceps* induced metamorphosis in 80% of larvae (Fig. 2a). Concentrations ≥10 µM of histamine (10, 50, 100 and 200 µM*)* were equally effective at inducing metamorphosis at 24 h (PERMANOVA—Pseudo $F_{(6,63)} = 22.04$, P-$_{MC} = 0.0001$, pair-wise comparisons P-$_{MC} > 0.05$). Histamine at 1 µM and FSW did not elicit a response from the larvae (Fig. 2a).

Species with planktotrophic larvae

Histamine did not induce metamorphosis in larvae of *Heliocidaris tuberculata* at any concentration tested (1, 10, 50, 100 and 200 µM). The geniculate coralline alga *Corallina officinalis* induced metamorphosis in 95% of larvae of *H. tuberculata* by 24 h. FSW did not induce any larval

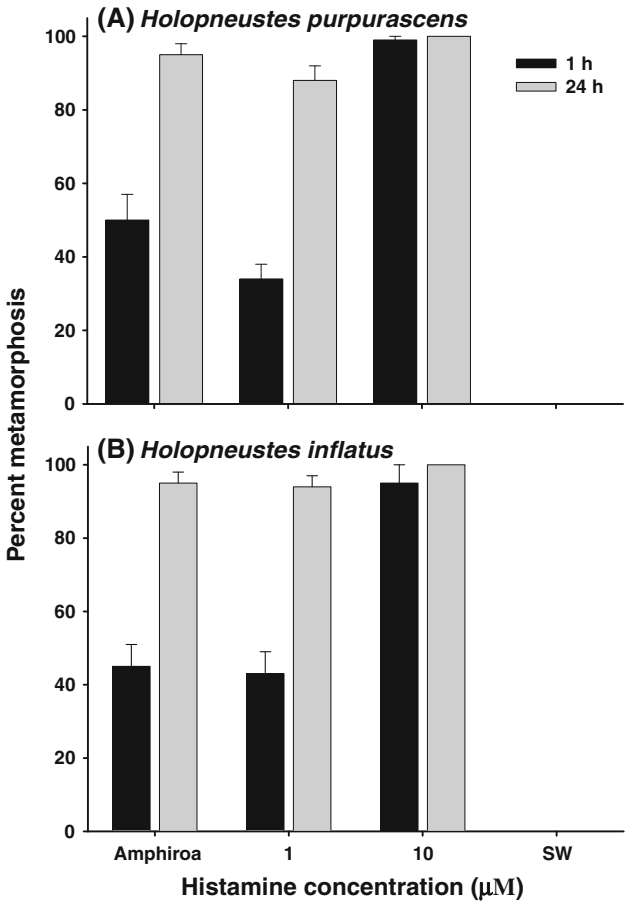

Fig. 1 Percent metamorphosis (mean ± SE) of larvae ($N = 10$) of **a** *Holopneustes purpurascens* and **b** *H. inflatus* after 1 h (*black bars*) and 24 h (*grey bars*) in response to the alga *Amphiroa anceps* (Amphiroa), histamine at 1 and 10 µM and sterile seawater (SW)

Table 3 Repeated measures ANOVA of the effects of species, histamine concentration and exposure time to histamine on metamorphosis of larval *Holopneustes purpurascens* and *H. inflatus*

Source	*df*	*F*	*P*
Between subjects			
Species	1	0.662	0.418
Concentration	4	2,152	<0.001
Species × concentration	4	0.227	0.923
Error	90		
Within subjects			G–G
Exposure time	2	158	<0.001
Exposure time × species	2	3.160	0.073
Exposure time × concentration	8	109	<0.001
Exp.time × species × conc.	8	2.145	0.072
Error	180		

Note that Greenhouse–Geisser (G–G)-adjusted *P*-values are used for the within subjects test as G–G $\varepsilon = 0.57$

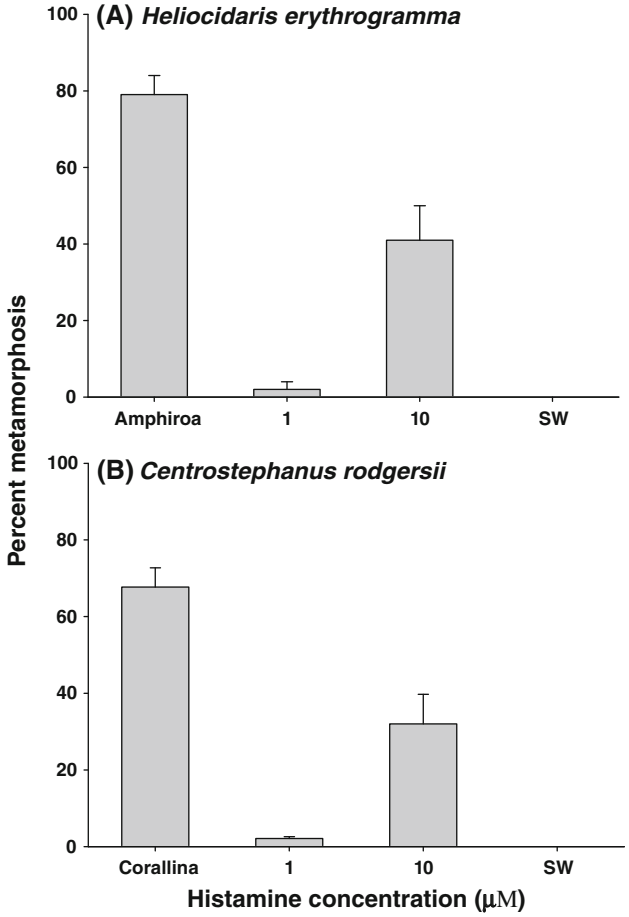

Fig. 2 Percent metamorphosis (mean ± SE) of larvae of **a** *Heliocidaris erythrogramma* ($N = 10$) and **b** *Centrostephanus rodgersii* ($N = 10$) after 24 h in response to algae (*Amphiroa anceps*) (Amphiroa) or *Corallina officinalis* (Corallina), histamine at 1 and 10 μM and filtered UV-sterilised seawater (SW). Note that response of *H. erythrogramma* larvae was also recorded after 1 h, and no larvae had metamorphosed in any treatment at this time

metamorphosis during the assay. While metamorphosis was not observed in response to histamine at 24–48 h, a significant proportion of larval *H. tuberculata* became attached to dishes containing 10–200 μM histamine. Between 12 and 20% of larval *H. tuberculata* were firmly attached to dishes at 24 h, which increased to 24–44% of larvae attached in dishes containing 100–200 μM of histamine at 48 h (PERMANOVA—Psuedo $F_{(5, 24)} = 6.729$, $P\text{-}_{MC} = 0.0003$; pair-wise comparison of 100/200 μM vs. FSW, $P\text{-}_{MC} < 0.05$). Histamine at 10–50 μM induced attachment of <10% of larvae by 48 h. Histamine at 1 μM and FSW did not induce attachment of larvae.

Histamine did not induce metamorphosis in larvae of *Tripneustes gratilla* at any concentration tested (0.1, 1, 10 and 100 μM). Approximately 30% of larval *T. gratilla* metamorphosed in response to *C. officinalis* by 48 h, indicating that a significant proportion of these larvae were not competent to metamorphose because this alga is a reliable cue. FSW did not induce any larval metamorphosis after 48 h.

Histamine at 10 μM induced metamorphosis in 30% of larvae of *Centrostephanus rodgersii,* and 100-μM histamine induced >95% of larvae to metamorphose by 24 h. Approximately 70% of larvae of *C. rodgersii* metamorphosed in response to *C. officinalis* by 24 h (Fig. 2b; PERMANOVA—Psuedo $F_{(4, 45)} = 111.2$, $P\text{-}_{perm} = 0.0001$; pair-wise comparison of 1/10 μM vs. FSW, $P\text{-}_{MC} < 0.001$)). Histamine at 1 μM and FSW induced minimal metamorphosis (<5%) of larvae by 24 h. A further 20% of larval *C. rodgersii* had metamorphosed in response to histamine (1 and 10 μM) and *C. officinalis* by 48 h.

Host plant assay with *Holopneustes* species

Larvae of *H. inflatus* and *H. purpurascens* were induced to metamorphose by all species of seagrass and algae assayed with varying efficacy (Fig. 3). Interestingly, rates of larval metamorphosis after 1 h favoured the host plants of each species. That is, more larvae of *H. purpurascens* (than *H. inflatus*) had metamorphosed in response to *D. pulchra* and *A. anceps* at 1 h, whereas more larvae of *H. inflatus* (than *H. purpurascens*) had metamorphosed in response to the three seagrasses at 1 h (Fig. 3a). The different responses of larvae to host plants that were apparent at 1 h (Fig. 3a) had diminished by 24 h (Fig. 3b) as indicated by the time x species x plant interaction ($P = 0.034$, Table 4). Although there was no effect of (larval) species in the analysis, there was a significant species x (host) plant interaction (Table 4) suggesting that larvae of *H. inflatus* and *H. purpurascens* were responding differently to host plants.

Algal assay with *Centrostephanus rodgersi*

All algal species assayed induced metamorphosis in larvae of *C. rodgersii* with the red algae inducing 35–68% metamorphosis by 24 h (Fig. 4; PERMANOVA—Psuedo $F_{(8, 81)} = 15.368$, $P\text{-}_{perm} = 0.0001$; pair-wise comparison of alga vs. FSW, each $P\text{-}_{MC} < 0.01$). Brown algae induced 9–31% metamorphosis in larvae of *C. rodgersii* by 24 h, while FSW had no effect on larvae.

Discussion

As documented previously for *Holopneustes purpurascens* (Swanson et al. 2004), histamine is also an effective metamorphic cue for the lecithotrophic larvae of *Holopneustes inflatus* (Temnopluridae) and *Heliocidaris erythrogramma* (Echinometridae), and the planktotrophic larvae of *Centrostephanus rodgersii* (Diadematidae). In contrast, histamine

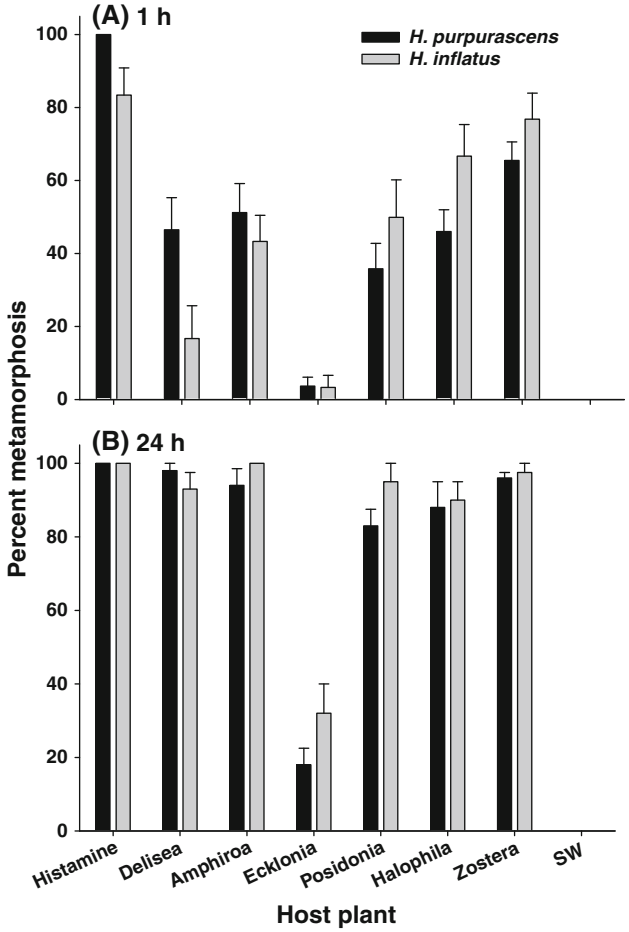

Fig. 3 Percent metamorphosis (mean ± SE) of larvae ($N = 10$) of *Holopneustes purpurascens* (*black bars*) and *H. inflatus* (*grey bars*) in response to histamine (10 μM), different host plants and sterile seawater (SW) after (**a**) 1 h and (**b**) 24 h. Delisea = *D. pulchra*, Amphiroa = *A. anceps*, Ecklonia = *E. radiata*, Posidonia = *P. australis*, Halophila = *H. ovalis,* Zostera = *Z. capricornia*

Table 4 Repeated measures ANOVA of the effects of species, host plant (seagrasses and algae) and exposure time to host plants on metamorphosis of larval *Holopneustes purpurascens* and *H. inflatus*

Source	*df*	*F*	*P*
Between subjects			
Species	1	1.776	0.186
Plant	5	54	<0.001
Species × plant	5	2.953	0.015
Error	108		
Within subjects			
Exposure time	1	304	<0.001
Exposure time × species	1	0.258	0.612
Exposure time × plant	5	9.724	<0.001
Exp.Time × species × plant	5	2.519	0.034
Error	108		

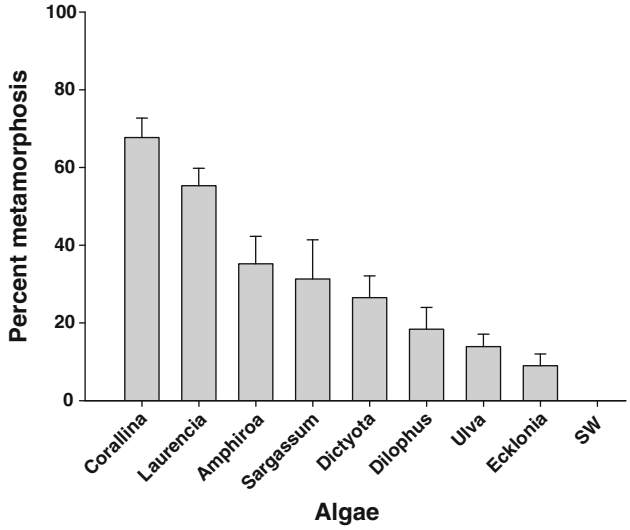

Fig. 4 Percent metamorphosis (mean ± SE) of larvae of *Centrostephanus rodgersii* ($N = 10$) in response to common algae from its habitat and filtered UV-sterilised seawater (SW) after 24 h. Corallina = *C. officinalis*, Laurencia = *Laurencia* sp., Amphiroa = *A. anceps*, *Sargassum* = *S. linearifolium*, Dictyota = *D. dichotoma*, Dilophus = *D. marginata*, Ulva = *Ulva* sp, Ecklonia = *E. radiata*

did not induce metamorphosis in the echinoplutei of *Heliocidaris tuberculata* (Echinometridae) and *Tripnesutes gratilla* (Toxopneustidae) although some larvae of *H. tuberculata* attached in response to histamine. Thus, the species that responded to histamine were a disparate assemblage of species with regards to developmental mode and phylogeny (Table 1). It is difficult to make direct comparisons of the larval response to histamine as different proportions of each larval species were competent to metamorphose during the assay. Competency is indicated by the number of larvae that metamorphosed in response to the coralline alga included in the assay (Table 2). The larval responses to selected algae suggest that 80–95% of larvae of the lecithotrophic species, and approximately two-third of the planktotrophic species *C. rodgersii,* were competent to metamorphose in the assays at 24 h. Histamine was most effective at inducing metamorphosis in larvae of the two *Holopneustes* species as 1-μM histamine induced metamorphosis in >90% of these larvae at 24 h, compared with ~2% of larvae of *H. erythrogramma* and *C. rodgersii*.

Habitat similarities among the species that responded to histamine may have influenced the pattern of response of larvae in laboratory assays. Three of the species that metamorphosed in response to histamine, that is, *H. purpurascens*, *H. erythrogramma* and *C. rodgersii,* co-occur in subtidal rocky reefs. We propose that dissolved histamine that leaches from algae and kelp, as shown for *Delisea pulchra* and *Ecklonia radiata* (Swanson et al. 2006; Tebben 2008), may act as a signpost of algal-dominated habitats to competent sea urchin larvae, providing a cue for an

appropriate site in which to settle and metamorphose. Peptides and amino acids leak from algae (Agrawal and Sharma 1996) and sea urchin larvae have been shown to use amino acids and sugars as signals for morphological change (Shilling 1995). Histamine may also leak from algae and serve as an exogenous cue for metamorphosis for sea urchin larvae. A quantitative survey of the histamine content of common algae from a typical habitat of *H. purpurascens*, *H. erythrogramma*, *H. tuberculata* and *C. rodgersii* confirmed the production of this naturally occurring metamorphic cue in all seasons (Swanson et al. 2006). Quantitative analysis of the histamine content of several species of red and brown algae showed that they contained 0.01–300 μg g^{-1} (dry weight) histamine (Swanson et al. 2006; Tebben 2008). Given that histamine comes from the decarboxylation of the amino acid histidine, it is probable that other species of red and brown algae also contain at least some level of histamine. Epiphytic growth of filamentous algae (fouling) on the surface of macroalgae generally increases during the warmer months (Hellio et al. 2004), and fouling taken from the laminae of *E. radiata* contained 100-fold higher concentrations of histamine than unfouled laminae of *E. radiata* (Swanson et al. 2006). Thus dense beds of *E. radiata* and *Sargassum* spp., as well as beds of *D. pulchra*, might provide a source of histamine in the habitat, particularly during the warmer months.

Heliocidaris tuberculata co-occurs with *H. purpurascens*, *H. erythrogramma* and *C. rodgersii* in algal-dominated habitats, but the larvae of this species only attached in response to histamine and did not metamorphose. The slow attachment of larvae in response to relatively high concentrations of histamine (10–200 μM) indicates a weak effect of histamine on larvae of *H. tuberculata.* Dissolved histamine may, however, influence settlement behaviour bringing the competent larvae of this species closer to metamorphic cues in the habitat. Serotonin and its precursor 5-hydroxytryptophan, invoke a negative phototaxis in larvae of the bryozoan *Bugula neritina* implying a neurochemical regulatory role for these biogenic amines in larval settlement (Pires and Woollacott 1997). *Heliocidaris tuberculata* may use separate cues for settlement and metamorphosis, as shown for a nudibranch *Onchidoris bilamellata* and a barnacle *Balanus amphitrite* (Chia and Koss 1988; Clare and Matsumura 2000). Low concentrations of dissolved histamine may be effective in nature, where synergistic effects of water flow and chemical cues affect the behaviour of settling larvae (Wright and Boxshall 1999; Alteri 2003).

Holopneustes inflatus inhabits seagrass beds, rather than the algal-dominated rocky reefs of its congener. Histamine induced rapid metamorphosis in 80% of larval *H. inflatus* and the seagrasses (*Posidonia australis, Halophila ovalis* and *Zostera capricornia*) induced >90% metamorphosis by 24 h (Fig. 3). It is not known whether *H. inflatus* recruits to seagrass beds in Botany Bay, but it seems likely, given that seagrasses produce a metamorphic cue for larval *H. inflatus*, and juvenile *H. inflatus* were found there in this study. Sea urchins are found in temperate, subtropical and tropical seagrass beds (Lawrence 1975) where they are among the most common macrograzers (Vergés et al. 2007; Eklöf et al. 2008). Larvae of both species of *Holopneustes* were induced to metamorphose by the favoured host plants of their congener albeit at slower rates initially. Seagrasses contain similar levels of histamine as brown algae (∼0.5 μg g^{-1} dw) based on semi-quantitative analyses (Swanson et al. 2006; Swanson 2007). The similar response of larval *H. inflatus* and *H. purpurascens* to histamine, seagrass and algae suggests that they respond to the same metamorphic cue that is likely to be histamine. Dense beds of seagrass may leach histamine into surrounding seawater, a potential signpost for seagrass beds for competent *H. inflatus* larvae. In this instance, the calmer conditions in which seagrass beds typically occur may lead to longer retention of dissolved histamine of seagrass origin (Krug and Zimmer 2000).

The different habitats occupied by adult *H. purpurascens*, *H. inflatus, H. erythrogramma* and *C. rodgersii* may influence their different larval sensitivities to histamine. *Holopneustes purpurascens* and *H. inflatus* are atypical echinoids in that they are specialists in habitat use and diet. That is, *H. purpurascens* and *H. inflatus* live in the canopy of their host plants, algae/kelp and seagrass, respectively, using it as habitat as well as food (Steinberg 1995; Williamson et al. 2004). Conversely, the life style of *H. erythrogramma* and *C. rodgersii* as generalists foraging on the sea floor (Hill et al. 2003; Ling et al. 2010) is more typical of urchins. Larvae of habitat specialists such as *H. purpurascens* and *H. inflatus*, which live on a discrete range of algae/plants, may have evolved a more sensitive and rapid response to cues from their host plants to enhance post-larval success. Generalist marine herbivores such as *H. erythrogramma* and *C. rodgersii,* on the other hand, are less restricted in their habitat use and diet and thus might be expected to have a lower specificity in their metamorphic cues (Huggett et al. 2006). *Centrostephanus rodgersii* demonstrated low specificity in its cues for metamorphosis by responding to a wide range of red, brown and green algae. Only ∼2% of larvae of *H. erythrogramma* and *C. rodgersii* metamorphosed in response to 1-μM histamine whereas most larvae of the *Holopneustes* species had metamorphosed by 24 h. This difference in sensitivity to histamine as metamorphic inducer may reflect the ability of *H. erythrogramma* and *C. rodgersii* larvae to respond to cues from a wide range of algae present in the habitat.

Histamine had no effect on metamorphosis in larvae of another generalist urchin *T. gratilla.* Even though only a

third of larval *T. gratilla* were competent to metamorphose in the assay, no effect of histamine (0.1, 1.0, 10 and 100 μM) was observed over 48 h, with similar results seen in preliminary assays. *Tripneustes gratilla* is a widely distributed tropical Indo-West Pacific species often found in habitats dominated by *Sargassum* spp. and seagrass (Juinio-Meñez and Bangi 2010). As a generalist, *T. gratilla* also appears to have low specificity in its cues for settlement and metamorphosis as these processes are triggered by a variety of algae, conspecifics and biofilms enriched with diatoms (Dworjanyn and Pirozzi 2008). When *Sargassum linearifolium* was cleaned to reduce the abundance of surface bacteria, the alga no longer induced metamorphosis of *T. gratilla* (Dworjanyn and Pirozzi 2008). These observations suggest a biofilm-derived metamorphic cue for *T. gratilla* and support the hypothesis of Steinberg et al. (2001) that larvae of generalist herbivores are likely to metamorphose in response to biofilms.

The mechanism by which histamine induces metamorphosis in sea urchin larvae may be twofold and may differ among species. The rapid metamorphic response of larvae of *Holopneustes* spp. to histamine suggests induction via an external histamine receptor, which is the expectation for a true metamorphic cue in nature. A sea urchin homologue of the metabotropic histamine H_1 receptor of vertebrates (suH_1R) has been identified on the surface of *Strongylocentrotus purpuratus* eggs, and this receptor is an integral part of the Ca^{2+} release pathway leading to fertilisation (Leguia and Wessel 2006). It is possible that suH_1R is expressed in other sea urchins and may be involved in histamine-induced metamorphosis of sea urchin larvae, but this hypothesis requires further investigation. Rather than only acting via an external receptor, histamine may also be an endogenous signalling molecule involved in the control of metamorphosis in sea urchin larvae. Dissolved histamine in the water may lead to an increase in endogenous levels of histamine over 24 h, triggering metamorphosis. Endogenous levels of the other biogenic amines (dopamine, serotonin) and hormones (thyroxine) appear to control or modulate competency and metamorphosis in hydrozoan, molluscan and echinoderm larvae (Pires et al. 2000; Leise et al. 2001; Pechenik et al. 2002; Heyland and Hodin 2004). Biogenic amines, all products of the decarboxylation of amino acids, are known to play critical roles in initiating and controlling behaviour and in the physiology of invertebrates, by acting as classical neurotransmitters, neuromodulators and neurohormones (Katz 1995). Thus, histamine may have a regulatory role in the settlement and metamorphosis of sea urchin larvae.

Under laboratory conditions, 10-μM histamine induced metamorphosis of larvae of *H. purpurascens, H. inflatus, H. erthyrogramma* and *C. rodgersii*. In nature, however, measured concentrations of histamine in seawater samples (10 mL) collected adjacent to *D. pulchra* were much lower, between 5 and 15 nM, while seawater collected at the sea surface contained 0.5-nM histamine (Swanson et al. 2006). Contrary to past assumptions, dissolved chemicals emanating from substrata do not form smooth concentration gradients (Crisp 1974). Rather, chemical cues are dispersed in fine filaments of high concentration swirling in clean water, where wave-driven flow generates wider filaments of higher concentration compared to unidirectional flow (Koehl 2006). Thus inductive concentrations of histamine (μM) may accumulate in such fine-scale filaments, at spatial scales (nm, μm) appropriate for larval detection (Hadfield and Koehl 2004) but may have been diluted in the 10-mL seawater samples that were analysed for histamine concentration (Swanson et al. 2006). This phenomenon of fine-scale spatial distribution of dissolved cues was shown to occur in the turbulent oscillatory flow above coral reefs in which larvae of *Phestilla sibogae* are transported (Hadfield and Koehl 2004). These larvae sank when encountering filaments of odour (containing a metamorphic cue) emanating from a coral reef exposed to wave action, and then resumed swimming in odour (cue)-free seawater (Hadfield and Koehl 2004). Larval behaviour of sinking in response to dissolved cues in the water column can increase the rate of larval transport to the substratum in turbulent wave-driven flow, thereby enhancing the rate of metamorphosis (Hadfield and Koehl 2004). Oyster larvae *Crassostrea virginica* in a flume boundary layer exhibited rapid downward acceleration, or "dive-bombing," in response to dissolved cues, a behaviour bringing them into contact with the substratum (Finelli and Wethey 2003). Larval behaviours in response to dissolved cues are increasingly recognised as important contributors to the recruitment patterns of marine invertebrates.

Increasing evidence suggests that histamine is a ubiquitous signalling molecule in the nervous systems of a diverse range of invertebrates and vertebrates (Reite 1972; Claiborne and Selverston 1984; Bayer et al. 1989; Stuart 1999; Zimmer and Zimmer 2008). Many chemical signals involved in neurotransmission or neuromodulation at the cellular level (e.g. amino acids, peptides and hormones) also function as external chemical signals in the aquatic environment and so act as both exogenous and endogenous signalling molecules (Haldane 1954; Carr 1988; Heyland et al. 2005; Heyland and Moroz 2005). Histamine may prove to be such a compound. Additional measurements of dissolved histamine in seawater surrounding algae/kelp, and in seagrass beds, are needed to address the hypothesis that histamine may be a naturally occurring metamorphic cue for larvae of sea urchins; *H. purpurascens, H. inflatus, H. erthrogramma* and *C. rodgersii*. The potential role of endogenous levels of histamine in regulating metamorphosis in sea urchin larvae is a worthy avenue for further research.

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