The Journal of Experimental Biology 210, 3228-3235
Published by The Company of Biologists 2007
doi:10.1242/jeb.004192

Larval desperation and histamine: how simple responses can lead to complex changes in larval behaviour

R. L. Swanson[1,*], D. J. Marshall[2] and P. D. Steinberg[1]

[1]*Centre for Marine Biofouling and Bioinnovation/School of Biological, Earth and Environmental Sciences, The University of New South Wales, New South Wales, 2052, Australia and* [2]*School of Integrative Biology/Centre for Marine Studies, University of Queensland, Queensland, 4072, Australia*

*Author for correspondence (e-mail: r.swanson@unsw.edu.au)

Accepted 23 June 2007

Summary

Some marine invertebrate larvae expand the range of settlement cues to which they will respond as they age. How do relatively simple larvae achieve such complex changes in behaviour? Larvae of the Australian sea urchin *Holopneustes purpurascens* settle and metamorphose specifically in response to a settlement cue, dissolved histamine, produced by the host alga *Delisea pulchra*. Older *H. purpurascens* larvae appear to accept a wider range of host algae, which contain far less histamine than *D. pulchra*, than newly competent larvae. We tested the hypothesis that older *H. purpurascens* larvae accept a greater range of host algae by metamorphosing in response to lower concentrations of histamine. We compared the response of newly competent and older larvae to a range of histamine concentrations in settlement assays. Larval age strongly affected the minimum concentration of histamine that induced metamorphosis in *H. purpurascens*, with older larvae responding to lower concentrations of histamine than newly competent larvae. Older larvae were more sensitive to lower concentrations of histamine yet still maintained a stringent requirement for exposure to histamine in order to metamorphose. In addition, older larvae metamorphosed after shorter exposure periods to histamine than did younger larvae. By using histamine concentration as a proxy for specific habitat cues, *H. purpurascens* larvae appear to expand their range of settlement preferences with age by simply changing their sensitivity to a single settlement cue. Overall, our results show that marine invertebrate larvae can exhibit surprisingly complex changes in behaviour *via* simple changes in their response to a single cue.

Key words: desperate larva hypothesis, settlement behaviour, metamorphosis, histamine, *Holopneustes purpurascens*.

Introduction

The majority of marine invertebrates have bi-phasic life histories: a planktonic larval phase that can last from hours to several months, followed by the benthic phase, initiated through larval settlement and metamorphosis to the juvenile form. Settlement and metamorphosis of larvae into an appropriate habitat are paramount to the survival of the juvenile/adult, particularly so for those benthic invertebrates that are sessile or sedentary after metamorphosis. Most larvae respond to chemical cues in the benthos during settlement, which trigger attachment and metamorphosis on favoured substrata (Hadfield and Paul, 2001; Mullineaux and Butman, 1991; Raimondi and Morse, 2000). In the absence of obligatory settlement cues, larval species from a range of phyla are able to postpone metamorphosis until such cues are encountered (Pechenik, 1990). This ability of some larvae to delay metamorphosis, despite being developmentally ready for the transition (or 'competent'), is believed to increase the chance of settling into habitats that can support survival to adulthood (Morgan, 1995; Pechenik, 1990; Thorson, 1950). Extending the planktonic phase, however, comes with an increased risk of larval mortality and can lead to detrimental carry-over effects for juvenile/adult performance (Maldonado and Young, 1999; Marshall et al., 2003; Pechenik et al., 1998; Wendt, 1998).

Lecithotrophic (non-feeding) larvae have limited energetic reserves to support their development during the planktonic phase and through metamorphosis, and so have a limited time to locate a favourable habitat to resume the benthic phase. Knight-Jones (Knight-Jones, 1953) and Wilson (Wilson, 1953) first noted that larvae become less discriminating with regard to habitat selection as they age. Thus, some species are presumed to accept a broader range of microhabitats as they age because they are approaching their energetic minimum and are therefore 'desperate' to settle and metamorphose anywhere. This view has subsequently been described as the 'desperate larva hypothesis' – or DLH (Toonen and Pawlik, 1994) – and, since then, the DLH has been used to explain the decreased substratum specificity observed in a range of older non-feeding larvae (Gribben et al., 2006; Marshall and Keough, 2003; Miron et al., 2000). More recently, the DLH has been modified to incorporate the effects of larval feeding and the consequences of habitat specialisation. Botello and Krug (Botello and Krug, 2006) have shown that for the opisthobranch *Alderia* sp., which feeds exclusively on the

alga *Vaucheria longicaulis* (Krug and Manzi, 1999; Krug and Zimmer, 2000), older larvae do not metamorphose indiscriminately. Rather, older, starved larvae of *Alderia* sp. became more sensitive to settlement cues from the host alga, but older, fed larvae did not (Botello and Krug, 2006). They suggest that the DLH should not apply to host-dependant species because metamorphosis in the absence of such hosts/prey would likely be fatal (Botello and Krug, 2006), and a theoretical consideration of the problem supports this suggestion (Elkin and Marshall, 2007).

Despite the increased interest in the DLH and its apparent applicability to a wide range of species (reviewed in Elkin and Marshall, 2007), we have little idea as to the mechanism of decreased selectivity with respect to settlement cues (i.e. 'desperation'). Desperate larvae are rarely indiscriminate; for example, larvae of the opisthobranch *Haminaea callidegenita* react to a greater variety of cues as they age; young larvae metamorphose in response to a single inducer but older larvae metamorphose in response to *Zostera marina* and a green alga but not biofilmed sediment (Gibson, 1995). How do relatively simple larvae increase the range of cues that they react to whilst continuing to reject other cues? To examine this question, we used the lecithotrophic larvae of the Australian sea urchin *Holopneustes purpurascens*. *H. purpurascens* is a relatively specialised herbivore that lives on and consumes kelp, *Ecklonia radiata*, during the adult benthic phase (Steinberg, 1995; Williamson et al., 2004). As a newly settled juvenile, it is found predominantly on the red alga *Delisea pulchra* and coralline turfing algae *Amphiroa anceps* and *Corallina officinalis* (Swanson et al., 2006). The observed distribution of new recruits in the field matched settlement choices in the laboratory, where larvae metamorphosed in response to *D. pulchra* and coralline algae but not *E. radiata* (Swanson et al., 2006). *H. purpurascens* larvae metamorphose specifically in response to dissolved histamine, a naturally occurring settlement cue produced in high quantities by the red foliose alga *D. pulchra* (Swanson et al., 2006; Swanson et al., 2004). The settlement cue(s) from coralline algae are unknown but seem to be produced by the surface-associated microbial community (biofilm) and may be bacterial histamine (Swanson et al., 2006).

H. purpurascens appears to be a typical species to which the DLH applies: red algae (*D. pulchra*, *A. anceps*, *C. officinalis*) are common hosts of new recruits of *H. purpurascens* (Swanson et al., 2006), and these algae induced 20–100% metamorphosis of newly competent (6-day-old) larval *H. purpurascens* (Williamson et al., 2004). Brown algae (*Sargassum vestitum*, *E. radiata*) induce minimal metamorphosis of newly competent larvae; however, as they age, their response to brown algae increases (Williamson et al., 2004). *S. vestitum* and *E. radiata* contain histamine but at much lower levels than the preferred host of new recruits, *D. pulchra* (Swanson et al., 2006). Thus, older *H. purpurascens* larvae may expand the range of host algae by metamorphosing in response to lower concentrations of histamine. Alternatively, they may be responding to different cues present in brown algae as they age and use entirely different response pathways. We sought to distinguish among these hypotheses.

We tested the hypothesis that older *H. purpurascens* respond to a greater range of host algae by reducing the threshold concentration of histamine required for metamorphosis using settlement bioassays. Three batches of larvae were tested against a range of concentrations of dissolved histamine, at competence and each week thereafter for 2–3 weeks, to see if older larvae (1) became more sensitive (responsive) to low concentrations of histamine and (2) showed decreased specificity for histamine as a settlement cue. Recent findings suggest that older larvae may require less exposure to settlement cues than younger larvae to induce metamorphosis (Jackson et al., 2005). Therefore, we also tested the hypothesis that older *H. purpurascens* required less exposure to histamine than newly competent larvae to induce metamorphosis by exposing larvae at 7, 14 and 21 days old (same batch) to 10 μmol l^{-1} histamine for different time periods.

Materials and methods

Larval culture

Adult *Holopneustes purpurascens* Agassiz 1872 (30–40) and ambient seawater (SW) were collected from Bare Island (Sydney, Australia) in July, August and October 2004. Sea urchins were held in a constant-temperature room (CTR) at 19°C in two buckets containing 15 litres of ambient seawater with continuous air in a 12 h:12 h light:dark cycle. Urchins were transferred to clean buckets with fresh ambient SW at least every other day and were maintained until they spawned and died. Buckets were checked each morning for spawn. Air was stopped to allow (fertilised) eggs to float to the surface, where they were gently collected in a 200 ml glass dish and poured into three autoclaved 2-litre beakers. Eggs were rinsed three times in sterilised SW containing antibiotics (22 g l^{-1} penicillin G, 37 g l^{-1} streptomycin sulfate, SSW) and were cultured in the CTR outlined above. Cultures were aerated continuously and SSW was changed every other day. Batches yielding sufficient numbers of hatched larvae (~2000) were kept for settlement assays over ensuing weeks. Each batch of larvae was of mixed parentage, with parents also differing between batches.

Settlement assays

All assays were done in the CTR in 36-mm sterile Petri dishes with 4 ml SSW under static conditions. Replicates were randomly assigned among treatments. Treatment and control dishes were prepared by first adding aliquots of concentrated stock solutions of histamine in SSW followed by the addition of SSW to bring histamine concentration to the desired test concentration. Larvae were added once all Petri dishes were prepared. Larval *H. purpurascens* reach competence (developmentally ready for metamorphosis, recognised by the presence of five well-developed tube-feet) at around 6 days old (6 days post fertilisation).

Dose–response of larvae of different ages

The dose–response of *H. purpurascens* larvae was investigated to see if older larvae become more sensitive to lower concentrations of histamine as an inducer of metamorphosis. Assays were done with three batches of larvae (A, B and C) at 7 days old (i.e. newly competent larvae), 14 days old (~1 week post initial-competence), 21 days old (~2 weeks

post initial-competence) and 28 days old (~3 weeks post initial-competence; batch A only). Histamine was tested at 1 μmol l^{-1}, 100 nmol l^{-1} and 10 nmol l^{-1} in each assay as an inducer of metamorphosis. Histamine was tested at 10 μmol l^{-1} as a positive control because this concentration induced maximal metamorphosis of larvae in previous studies (Swanson et al., 2004; Swanson et al., 2006). SSW was tested in all assays as a measure of spontaneous metamorphosis. Twenty-five larvae from batches A and B were used per treatment (five dishes with five larvae), and 100 larvae from batch C were used per treatment (10 dishes with 10 larvae). Percent metamorphosis was scored at 1, 24, 48 and 72 h.

A repeated-measures ANOVA was used to examine the effects of larval age on the sensitivity of larvae to lower concentrations of histamine as an inducer of metamorphosis (SYSTAT® 10.0 for Windows). Only histamine concentrations of 1 μmol l^{-1}, 100 nmol l^{-1} and 10 nmol l^{-1} were included in the analysis because 10 μmol l^{-1} histamine induced maximal metamorphosis and SSW induced minimal metamorphosis, regardless of larval age. Histamine was treated as a categorical factor rather than a co-variate because of the small range of values but the outcome of the analysis was the same in either case (D.J.M., unpublished analysis). Finally, values were pooled across all three experimental runs (i.e. batches A, B and C) with 7-day-old, 14-day-old and 21-day-old larvae for the analysis. We also applied the alternative approach of analysing each run separately, but the results were not qualitatively different and we therefore opted for the most parsimonious approach of pooling batches. Note that we first tested for interactions between run and the factors of interest but, as these were non-significant and of no biological interest, they were omitted from the final model following Quinn and Keough (Quinn and Keough, 2002). Data from batch A were analysed separately to include the response of 28-day-old larvae. For both analyses, Greenhouse-Geisser adjusted *P*-values were used as $\epsilon<0.75$ (Quinn and Keough, 2002). The use of Greenhouse-Geisser *P*-values represents a conservative approach that takes into account the fact that the assumption of 'sphericity' for the repeated-measures ANOVA was not met in our analyses [Quinn and Keough (Quinn and Keough, 2002), p. 337].

Effect of duration of exposure to histamine on inducing metamorphosis

H. purpurascens larvae were exposed to 10 μmol l^{-1} histamine for varying time periods at 7 days old, 14 days old and 21 days old to determine the duration of exposure that was required to induce settlement, a reversible process, and metamorphosis, an irreversible process, in larvae of different ages. Histamine was tested at 10 μmol l^{-1} in these experiments as this concentration induced maximal settlement/metamorphosis of larvae in previous studies (Swanson et al., 2004; Swanson et al., 2006). Herein, settlement is defined as the attachment of larvae to a surface *via* tube-feet, and metamorphosis is defined as the morphological transformation to the juvenile form. It was noted that attachment of *H. purpurascens* larvae immediately triggered the retraction of the larval body and the partial extrusion of juvenile spines and podia from the vestibule. However, these initial morphological changes in settled larvae were potentially reversible if they were removed from histamine exposure and placed in SSW. That is, a proportion of settled larvae reverted back to swimming larvae when placed in SSW depending on the duration of histamine exposure.

Seven-day-old larvae were exposed to 10 μmol l^{-1} histamine for 15 min, 20 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h and 5 h, and percent settlement was scored before transferring individuals to SSW. Percent metamorphosis of individuals was scored (in SSW) 24 h after commencing the experiment. Larvae were continuously exposed to 10 μmol l^{-1} histamine or SSW for 24 h in control treatments. A further set of procedural control dishes was included, transferring larvae from 10 μmol l^{-1} histamine dishes to another set of 10 μmol l^{-1} histamine dishes at each exposure time, to determine if the transfer process alone affected the metamorphosis of larvae. The assay was repeated with 14-day-old and 21-day-old larvae; however, not all exposure times could be tested due to limited numbers of larvae. Larvae that were 14 days old were exposed to 10 μmol l^{-1} histamine for 1 h, 2 h, 3 h, 4 h and 5 h, while 21-day-old larvae were exposed to histamine for 30 min, 45 min, 1 h and 2 h (shorter times were selected as the majority of older larvae were metamorphosing after 1 h exposure). Fifty larvae were used for each treatment (five dishes with 10 larvae). From these data, a 'reversion score' was calculated, which is the difference between the proportion of larvae that were metamorphosed at 24 h (in SSW) and the proportion of larvae deemed to have settled during exposure to 10 μmol l^{-1} histamine, before transferring to SSW. Thus, a positive score indicates that all larvae that had settled during exposure to histamine metamorphosed after transfer to SSW and that additional larvae also metamorphosed in the 24 h period. Conversely, a negative score indicates that a proportion of larvae that had settled during histamine exposure did not metamorphose after transfer to SSW but reverted back to swimming larvae. A two-factor (age = fixed factor, exposure time = fixed factor) analysis of variance (ANOVA) was used to examine the effects of larval age and exposure time on the induction of metamorphosis in *H. purpurascens*. The 'reversion scores' of newly competent larvae (7 days old) and older larvae (21 days old) in the 30 min, 45 min, 1 h and 2 h exposure treatments were compared; only these treatments were analysed as not all exposure times were tested with larvae of different ages.

Results

Dose–response of larvae of different ages

The percentage of larvae that metamorphosed in response to the low-range concentrations of 10 and 100 nmol l^{-1} histamine increased proportionally with larval age. That is, more 28-day-old larvae metamorphosed in response to 10 and 100 nmol l^{-1} histamine than 21-day-old larvae after 24, 48 and 72 h; more 21-day-old larvae metamorphosed in response to 10 and 100 nmol l^{-1} histamine than 14-day-old larvae after 24, 48 and 72 h; and more 14-day-old larvae metamorphosed in response to 10 and 100 nmol l^{-1} histamine than 7-day-old larvae (Fig. 1A–C). Only the oldest larvae (28 days old) metamorphosed in response to 10 nmol l^{-1} histamine after 1 h (Fig. 1A). There was minimal spontaneous metamorphosis in SSW during the assays (Fig. 1A–C). Between 30–40% of 7-day-

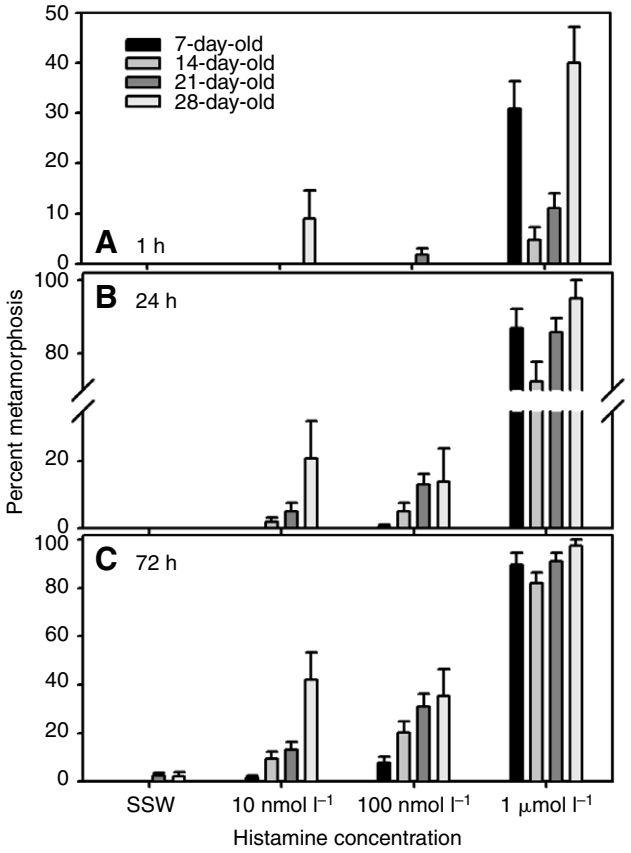

Fig. 1. Percent metamorphosis of *Holopneustes purpurascens* larvae (mean ± s.e.m.) of different ages after continuous exposure to 10 nmol l^{-1}, 100 nmol l^{-1} and 1 μmol l^{-1} dissolved histamine and SSW for 1 h (A), 24 h (B) and 72 h (C). Data from 7-day-old, 14-day-old and 21-day-old larvae were pooled from three batches (N=150 larvae per treatment); however, data shown for 28-day-old larvae are from batch A only (N=25 larvae per treatment).

old and 28-day-old larvae metamorphosed after 1 h exposure to 1 μmol l^{-1} histamine; however, less than 10% of 14-day-old and 21-day-old larvae metamorphosed rapidly to this concentration (Fig. 1A). After 24 h, over 70% of larvae of all ages had metamorphosed in response to 1 μmol l^{-1} (Fig. 1B). Most larvae (>85%) of all ages had metamorphosed in response to 10 μmol l^{-1} histamine after 1 h, which increased to 100% metamorphosis after 24 h.

Larval age strongly affected the minimum concentration of histamine that induced metamorphosis in *H. purpurascens*, with older larvae responding to lower concentrations of histamine than newly competent larvae (Table 1, Fig. 1). Older larvae metamorphosed in greater numbers than newly competent larvae in response to low concentrations of histamine (Table 1, Fig. 1). Because batch A had more time periods (7-day-old, 14-day-old, 21-day-old and 28-day-old) than the other batches, we also analysed this batch separately to include the response of 28-day-old larvae (Table 2). Older larvae from batch A also metamorphosed at lower concentrations than newly competent larvae (Fig. 1); however, in the analysis, this interaction was obscured by a significant time × age × concentration interaction (Table 2).

Effect of duration of exposure to histamine on inducing metamorphosis

Larval age strongly affected the induction of metamorphosis of *H. purpurascens* larvae, with older larvae requiring less exposure to 10 μmol l^{-1} histamine than younger larvae to induce metamorphosis (Table 3). Over 60% of newly competent larvae (7 days old) had settled after 20 min exposure to 10 μmol l^{-1} histamine (Fig. 2A). However, most settled larvae reverted back to swimming larvae once removed to SSW when exposed to histamine for 1 h or less, as indicated by the negative reversion scores (approximately –0.6) (Fig. 3A). Following 2 h exposure to 10 μmol l^{-1} histamine, approximately half of the settled larvae metamorphosed into juveniles after transfer to SSW. Newly competent larvae required 3 h continuous exposure to 10 μmol l^{-1} histamine to induce metamorphosis of >90% of settled larvae after transfer to SSW (Fig. 2A, Fig. 3A). Conversely, most 14-day-old larvae that had settled during 1 h (or longer) exposure to 10 μmol l^{-1} histamine metamorphosed after transfer to SSW, generating reversion scores close to zero (Fig. 2B, Fig. 3B). Although fewer 21-day-old larvae overall had settled after 30–45 min exposure to 10 μmol l^{-1} histamine, most settled larvae metamorphosed after transfer to SSW (Fig. 2C, Fig. 3C). Larvae of all ages that were continuously exposed to 10 μmol l^{-1} histamine were metamorphosed after 24 h. None of the 7-day-old or 14-day-old larvae had metamorphosed in control SSW after 24 h; however, approximately 10% of 21-day-old larvae had metamorphosed in control SSW after 24 h. All larvae in the procedural control treatment (transferred from 10 μmol l^{-1} histamine to 10 μmol l^{-1} histamine) were metamorphosed.

Discussion

Larval age strongly affected the minimum concentration of histamine that induced metamorphosis in *H. purpurascens*, with older larvae metamorphosing in response to much lower concentrations than younger larvae. Our study is the first of its kind to directly examine the effect of a pure form of a naturally occurring settlement cue on the dose–response curve of metamorphosis in young *versus* older larvae. This study supports the observations of Botello and Krug, who found that older larvae were more sensitive to lower concentrations of settlement cue (present in extracts of the alga *V. longicaulis*) than younger larvae (Botello and Krug, 2006). *H. purpurascens* joins a growing list of species that show shifts in the response of larvae as they age (reviewed in Elkin and Marshall, 2007). Given that *H. purpurascens* recruits are found on a number of different algae (Swanson et al., 2006), it seems reasonable to assume that this species, whilst having settlement preferences, is not a strict specialist at settlement. Thus, whilst larvae that settle and metamorphose on preferred host algae may have higher fitness, larvae that settle and metamorphose on other algae (i.e. brown algae) in the habitat may still survive. The shift to a broader range of host algae to facilitate metamorphosis of aging non-feeding larvae is predicted by a theoretical model (Elkin and Marshall, 2007). As larvae accumulate direct (planktonic mortality) and indirect (reduced energetic stores) costs in the plankton, the benefits of metamorphosing in a sub-optimal habitat increase (Elkin and Marshall, 2007).

Despite older *H. purpurascens* larvae reacting to lower and

Table 1. *Repeated-measures ANOVA of the effects of larval age (at competence, 1 week and 2 weeks post-competence), histamine concentration (excluding 0 and 10 μmol l^{-1}) and duration of exposure to histamine on induction of metamorphosis of Holopneustes purpurascens*

Source	d.f.	M.S.	*F*	*P*
Between subjects				
Concentration	2	304506	778.754	<0.001
Age	2	3340	8.542	<0.001
Concentration × age	4	4249	10.868	<0.001
Error	171	391		
Within subjects*				
Exposure time	3	40126	396.1	<0.001
Exposure time × concentration	6	19146	189.0	<0.001
Exposure time × age	6	982	9.7	<0.001
Exposure time × age × concentration	12	57	0.6	0.791
Error	513	101		

Data are pooled across batches A, B and C.
*Greenhouse–Geisser adjusted *P*-values are used for the within-subjects test as ϵ=0.62.

Table 2. *Repeated-measures ANOVA of the effects of larval age, histamine concentration and duration of exposure to histamine on induction of metamorphosis of Holopneustes purpurascens*

Source	d.f.	M.S.	*F*	*P*
Between subjects				
Concentration	1	220164	321.2	<0.001
Age	3	3963	5.8	0.002
Concentration × age	3	1225	1.8	0.161
Error	51	685		
Within subjects*				
Exposure time	3	1590	13.6	<0.001
Exposure time × concentration	3	11714	100.4	<0.001
Exposure time × age	9	420	3.6	0.007
Exposure time × age × concentration	9	367	3.2	0.014
Error	153	116		

Data are for batch A only with additional time period of three weeks post-metamorphosis.
*Greenhouse–Geisser adjusted *P*-values are used for the within-subjects test as ϵ=0.52.

lower concentrations of histamine, only a very small proportion (<5%) of very old larvae (28-day-old) metamorphosed spontaneously in the absence of any algal cues. Thus, older larvae do not metamorphose indiscriminately but become more sensitive to the settlement cue. Given that histamine is found at lower concentrations in other algae in the habitat, this increased sensitivity is likely to result in older larvae accepting a broader range of host algae. Our results appear therefore to be a novel example of expanding settlement preferences by becoming more responsive to a single settlement cue, which can occur here because of quantitative differences in the concentration of histamine among algae in the field (Swanson et al., 2006). By using histamine concentration as a proxy for a general habitat cue, complex changes in the settlement behaviour of older larvae can result from a simple change in their response to a single cue.

Table 3. *Two-factor ANOVA examining the effects of larval age (7 days old and 21 days old) and duration of exposure to histamine on the induction of metamorphosis of Holopneustes purpurascens*

Source	d.f.	M.S.	*F*	*P*
Age	1	3.691	146.2	<0.001
Exposure time	3	0.017	0.663	0.581
Age × exposure time	3	0.054	2.122	0.117
Error	32	0.025		

A number of studies have found that older polychaete, gastropod and abalone larvae metamorphose faster than younger larvae (Barlow, 1990; Botello and Krug, 2006; Knight-Jones, 1953; Pechenik and Cerulli, 1991). Similarly, older larvae of *Haliotis asinina* metamorphosed after shorter exposure periods to inductive algae than did younger larvae (Jackson et al., 2005). *H. purpurascens* larvae settled rapidly when exposed to 10 μmol l^{-1} histamine regardless of age (R.L.S., personal observation). Some morphological changes were evident in settled larvae such as the retraction of the larval body and the partial extrusion of juvenile spines and podia from the vestibule (R.L.S., personal observation). These initial changes in morphology were reversible in a proportion of younger larvae when exposed to histamine for less than 3 h, which suggests that components of the morphogenetic pathway were not activated sufficiently to trigger metamorphosis. Over

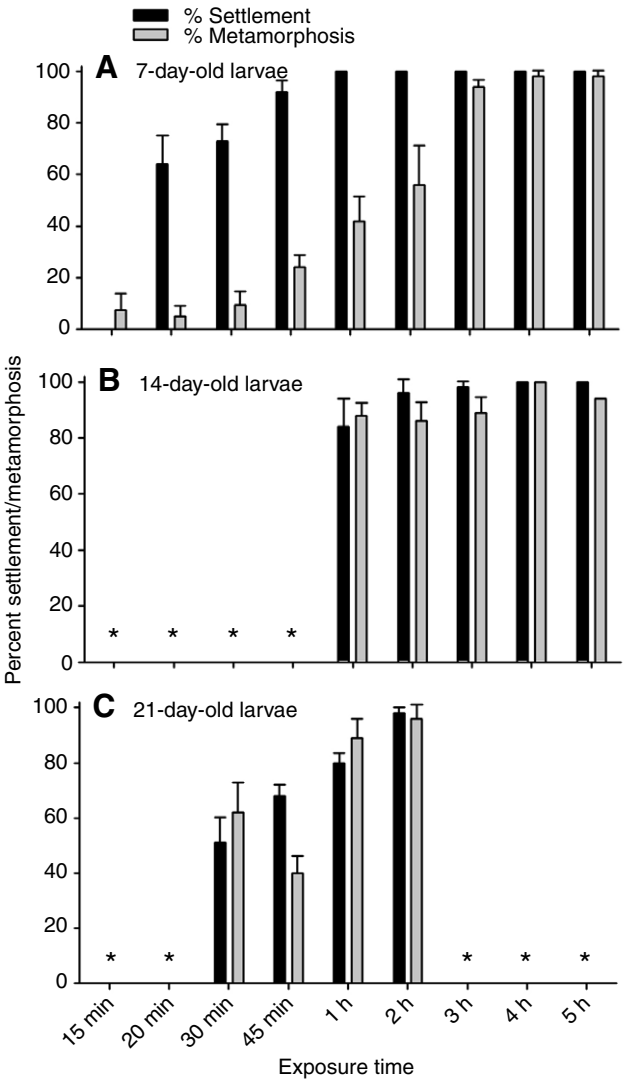

Fig. 2. Percent settlement of *Holopneustes purpurascens* larvae (mean ± s.e.m.; *N*=50 larvae per treatment) during exposure to 10 μmol l^{-1} histamine for varying time periods (black bars) and percent metamorphosis in SSW, 24 h after commencing the experiment (grey bars). The responses of larvae at 7 days old (A), 14 days old (B) and 21 days old (C) are shown. *, not tested.

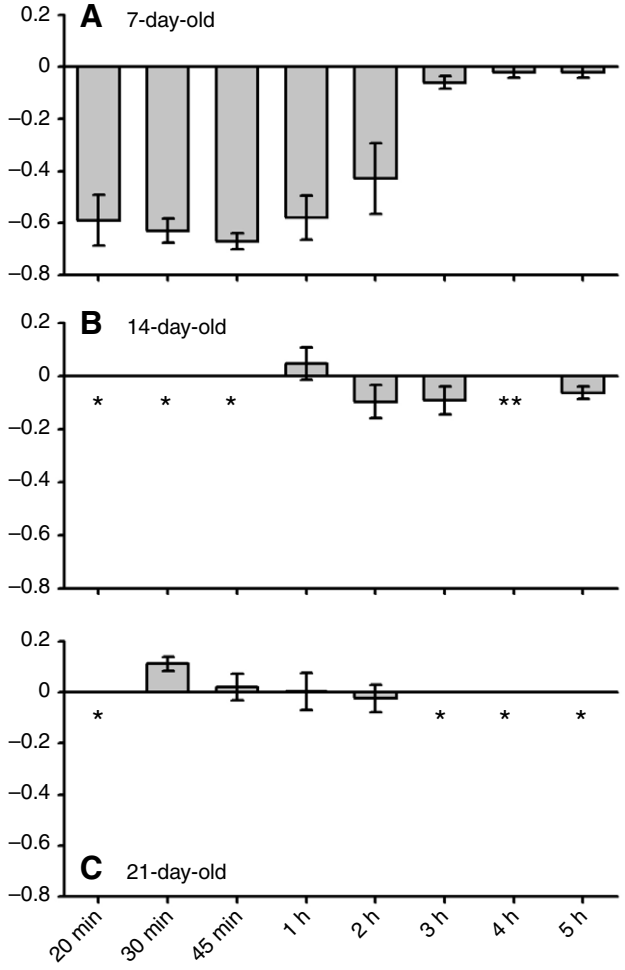

Fig. 3. The reversion scores of (A) 7-day-old, (B) 14-day-old and (C) 21-day-old *Holopneustes purpurascens* larvae after exposure to 10 μmol l^{-1} histamine for varying time periods. The reversion score was calculated as the difference between the proportion of larvae that had metamorphosed in SSW, 24 h after commencing the histamine exposure, and the proportion of larvae deemed to have settled during exposure to 10 μmol l^{-1} histamine before transfer to SSW. Thus, a negative score indicates that a proportion of settled larvae reverted back to swimming larvae after transfer to SSW. A positive score indicates that all settled larvae metamorphosed in SSW along with additional larvae over 24 h. *, not tested; **, all settled larvae metamorphosed.

90% of 7-day-old larvae had settled after 45 min exposure to 10 μmol l^{-1} histamine; however, most of these reverted back to swimming larvae when placed in SSW. Newly competent larvae required 3 h of continuous exposure to 10 μmol l^{-1} histamine to induce metamorphosis of all settled larvae. On the other hand, 21-day-old larvae required less than 1 h of exposure to 10 μmol l^{-1} histamine to trigger metamorphosis of all settled larvae. Thus, older larvae are induced to metamorphose soon after exposure to the settlement cue whereas younger larvae appear to be more flexible at the time of settlement. Newly competent *H. purpurascens* larvae that settle on an alga have the potential to reject the site for a limited period after attachment if the settlement cue is no longer perceived [for an alternative process in ascidian larvae, see Jacobs et al. (Jacobs et al., 2006)].

The increased sensitivity of *H. purpurascens* larvae to histamine occurred gradually with age, suggesting that there is a progressive decrease in the stimulus-threshold required to induce an aging larva to metamorphose, as noted for other invertebrate larvae (Coon et al., 1990; Crisp, 1974; Gibson, 1995; Knight-Jones, 1953; Pechenik, 1980; Rittschof et al., 1984). The mechanisms in older larvae that lead to increased sensitivity to histamine and a reduction in exposure time required for metamorphosis are unclear but are likely to have the same basis. Jackson et al. refer to 'competence factors' reaching a critical level in order for larvae to attain competency (Jackson et al., 2005). These factors may include chemoreceptors (Trapido-Rosenthal and Morse, 1986), components of internal signalling pathways (Clare et al., 1995; Knight et al., 2000) or transcription factors (Jackson et al.,

2005). These competence factors may continue to accumulate in older larvae, meaning they are 'primed' to respond to settlement cues, both to lower concentrations and more quickly than younger larvae (Jackson et al., 2005). For example, changes in endogenous levels of neurotransmitters may affect sensitivity to cues.

Catecholamines, such as dopamine and its precursor L-DOPA, appear to modulate competency and control metamorphosis in the gastropods *Crepidula fornicata* (Pechenik et al., 2002; Pires et al., 2000b) and *Phestilla sibogae* (Pires et al., 2000a). Increasing the endogenous dopamine concentrations of competent *P. sibogae* larvae made them more sensitive to the settlement cue present in coral extract [*Porites compressa* (Pires et al., 2000a)]. In the gastropod *Ilyanassa obsoleta*, metamorphosis only occurs following the removal of endogenous levels of nitric oxide (NO), a specific inhibitor of metamorphosis (Leise et al., 2001). Bath application of serotonin induced metamorphosis of *I. obsoleta* but not in the presence of two NO donors, and metamorphosis of *I. obsoleta* was induced, in the absence of serotonin or any other inducer, when endogenous NO production was inhibited (Leise et al., 2001). Other studies suggest that internal energy reserves also affect the sensitivity to settlement cues. Botello and Krug found that unfed larvae were more sensitive to settlement cues than fed larvae (Botello and Krug, 2006), and Marshall and Keough found that larger larvae (with more nutritional reserves) can delay settlement in the absence of cues for longer than smaller larvae (Marshall and Keough, 2003). Ultimately, each of these factors may also contribute to the increased sensitivity of older *H. purpurascens* larvae to histamine. However, it seems that the expansion of settlement preferences in older *H. purpurascens* larvae (Williamson et al., 2004) is based on simple changes in their response to a single settlement cue.

We wish to thank Patrick Krug and an anonymous reviewer for helpful comments that greatly improved the manuscript. This research was supported by an Australian Postgraduate Award to R.L.S. and Australian Research Council funding to D.J.M. and P.D.S.

References

Barlow, L. A. (1990). Electrophysiological and behavioral responses of larvae of the red abalone (*Haliotis rufescens*) to settlement-inducing substances. *Bull. Mar. Sci.* **46**, 537-554.

Botello, G. and Krug, P. J. (2006). Desperate larvae revisited: age, energy and experience affect sensitivity to settlement cues in larvae in the gastropod *Alderia* sp. *Mar. Ecol. Prog. Ser.* **312**, 149-159.

Clare, A. S., Thomas, R. F. and Rittschof, D. (1995). Evidence for the involvement of cyclic AMP in the pheromonal modulation of barnacle settlement. *J. Exp. Biol.* **198**, 655-664.

Coon, S. L., Fitt, W. K. and Bonar, D. B. (1990). Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. *Mar. Biol.* **106**, 379-387.

Crisp, D. J. (1974). Factors influencing the settlement of marine invertebrate larvae. In *Chemoreception in Marine Organisms* (ed. P. T. Grant and A. M. Mackie), pp. 177-265. London: Academic Press.

Elkin, C. and Marshall, D. J. (2007). Desperate larvae: the influence of deferred costs and habitat requirements on habitat selection. *Mar. Ecol. Prog. Ser.* **336**, 143-153.

Gibson, G. (1995). Why be choosy? Temporal changes in larval sensitivity to several naturally-occurring metamorphic inducers in the opistobranch *Haminaea callidegenita*. *J. Exp. Mar. Biol. Ecol.* **194**, 9-24.

Gribben, P. E., Marshall, D. J. and Steinberg, P. D. (2006). Less inhibited with age? Larval age modifies responses to natural settlement inhibitors. *Biofouling* **22**, 101-106.

Hadfield, M. G. and Paul, V. J. (2001). Natural chemical cues for settlement and metamorphosis of marine invertebrate larvae. In *Marine Chemical Ecology* (ed. J. B. McClintock and B. J. Baker), pp. 431-461. Florida: CRC Press.

Jackson, D. J., Ellemor, N. and Degnan, B. M. (2005). Correlating gene expression with larval competence, and the effect of age and parentage on metamorphosis in the tropical abalone *Haliotis asinina*. *Mar. Biol.* **147**, 681-697.

Jacobs, M. W., Degnan, S., Woods, R., Williams, E., Roper, K. E., Green, K. and Degnan, B. M. (2006). The effect of larval age on morphology and gene expression during ascidian metamorphosis. *Integr. Comp. Biol.* **46**, 760-776.

Knight, J., Rowley, A. F., Yamazaki, M. and Clare, A. S. (2000). Eicosanoids are modulators of larval settlement in the barnacle, *Balanus amphitrite*. *J. Mar. Biol. Assoc. U.K.* **80**, 113-117.

Knight-Jones, E. W. (1953). Decreased discrimination during settling after a prolonged planktonic life in larvae of *Spirorbis borealis* (Serpulidae). *J. Mar. Biol. Assoc. U.K.* **32**, 337-345.

Krug, P. J. and Manzi, A. E. (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. and Zimmer, R. K. (2000). Developmental dimorphism and expression of chemosensory-mediated behavior: habitat selection by a specialist marine herbivore. *J. Exp. Biol.* **203**, 1741-1754.

Leise, E. M., Thavaradhara, K., Durham, N. R. and Turner, B. E. (2001). Serotonin and nitric oxide regulate metamorphosis in the marine snail *Ilyanassa obsoleta*. *Am. Zool.* **41**, 258-267.

Maldonado, M. and Young, C. M. (1999). Effects of the duration of larval life on postlarval stages of the demosponge *Sigmadocia caerulea*. *J. Exp. Mar. Biol. Ecol.* **232**, 9-21.

Marshall, D. J. and Keough, M. J. (2003). Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Mar. Ecol. Prog. Ser.* **255**, 145-153.

Marshall, D. J., Pechenik, J. A. and Keough, M. J. (2003). Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial ascidian *Diplosoma listerianum*. *Mar. Ecol. Prog. Ser.* **246**, 153-162.

Miron, G., Walters, L. J., Tremblay, R. and Bourget, E. (2000). Physiological condition and barnacle larval behavior: a preliminary look at the relationship between TAG/DNA ratio and larval substratum exploration in *Balanus amphitrite*. *Mar. Ecol. Prog. Ser.* **198**, 303-310.

Morgan, S. G. (1995). Life and death in the plankton: larval mortality and adaptation. In *Ecology of Marine Invertebrate Larvae* (ed. L. R. McEdward), pp. 279-321. New York: CRC Press.

Mullineaux, L. S. and Butman, C. A. (1991). Initial contact, exploration and attachment of barnacle (*Balnanus amphitrite*) cyprids settling in flow. *Mar. Biol.* **110**, 93-103.

Pechenik, J. A. (1980). Growth and energy balance during the larval lives of three prosobranch gastropods. *J. Exp. Mar. Biol. Ecol.* **44**, 1-28.

Pechenik, J. A. (1990). Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? *Ophelia* **32**, 63-94.

Pechenik, J. A. and Cerulli, T. R. (1991). Influence of delayed metamorphosis on survival, growth and reproduction of the marine polychaete, *Capitella* sp. *J. Exp. Mar. Biol. Ecol.* **151**, 17-27.

Pechenik, J. A., Wendt, D. E. and Jarrett, J. N. (1998). Metamorphosis is not a new beginning. *Bioscience* **48**, 901-910.

Pechenik, J. A., Li, W. and Cochrane, D. E. (2002). Timing is everything: the effects of putative dopamine antagonists on metamorphosis vary with larval age and experimental duration in the prosobranch gastropod *Crepidula fornicata*. *Biol. Bull.* **202**, 137-147.

Pires, A. R., Croll, R. P. and Hadfield, M. G. (2000a). Catecholamines modulate metamorphosis in the opistobranch gastropod *Phestilla sibogae*. *Biol. Bull.* **198**, 319-331.

Pires, A. R., Guilbault, T. R., Mitten, J. V. and Skiendzielewski, J. A. (2000b). Catecholamines in larvae and juveniles of the prosobranch gastropod, *Crepidula fornicata*. *Comp. Biochem. Physiol.* **127C**, 37-47.

Quinn, G. P. and Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.

Raimondi, P. T. and Morse, A. N. C. (2000). The consequences of complex larval behaviour in a coral. *Ecology* **81**, 3193-3211.

Rittschof, D., Branscomb, E. S. and Costlow, J. D. (1984). Settlement and behaviour in relation to flow and surface in larval barnacles, *Balanus amphitrite*, Darwin. *J. Exp. Mar. Biol. Ecol.* **82**, 131-146.

Steinberg, P. D. (1995). Interaction between the canopy dwelling echinoid *Holopneustes purpurescens* and its host kelp *Ecklonia radiata*. *Mar. Ecol. Prog. Ser.* **127**, 169-181.

Swanson, R. L., Williamson, J. E., de Nys, R., Kumar, N., Bucknall, M. P.

and 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.

Swanson, R. L., de Nys, R., Huggett, M. J., Green, J. K. and Steinberg, P. (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.

Thorson, G. (1950). Reproduction and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**, 1-45.

Toonen, R. J. and Pawlik, J. R. (1994). Foundations of gregariousness. *Nature* **370**, 511-512.

Trapido-Rosenthal, H. G. and Morse, D. E. (1986). Regulation of receptor-mediated settlement and metamorphosis in larvae of a gastropod mollusc (*Haliotis rufescens*). *Bull. Mar. Sci.* **39**, 383-392.

Wendt, D. E. (1998). Effect of larval swimming duration on growth and reproduction of *Bugula neritina* (Bryozoa) under field conditions. *Biol. Bull.* **195**, 126-135.

Williamson, J. E., Carson, D. G., de Nys, R. and Steinberg, P. D. (2004). Demographic consequences of an ontogenetic shift by a sea urchin in response to host plant chemistry. *Ecology* **85**, 1355-1371.

Wilson, D. P. (1953). The settlement of *Ophelia bicornis* Savigny larvae. The 1951 experiments. *J. Mar. Biol. Assn UK* **31**, 413-438.