

Host location in flow by larvae of the symbiotic barnacle *Trevathana dentata* using odour-gated rheotaxis

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The detection and location of specific organisms in the aquatic environment, whether they are mates, prey or settlement sites, are two of the most important challenges facing aquatic animals. Large marine invertebrates such as lobsters have been found to locate specific organisms by navigating in the plume of chemicals emitted by the target. However, active plume tracking in flow by small organisms such as marine larvae has received little scientific attention. Here, we present results from a study examining host location in flow by nauplius larvae of the barnacle *Trevathana dentata*, which inhabits the stony reef coral *Cyphastrea chalcidicum*. The experiments included analysis of larval motion in an annular flume under four conditions: (i) still water, (ii) in flow, (iii) in still water with waterborne host metabolites and (iv) in flow with host metabolites. Our results show that *T. dentata* nauplii are unable to locate their target organism in still water using chemotaxis, but are capable of efficient host location in flow using odour-gated rheotaxis. This technique may enable host location by earlier, less-developed larval stages.

Keywords: chemotaxis; rheotaxis; host location; flow; invertebrate; larva

1. INTRODUCTION

The detection and location of specific organisms in the aquatic environment, whether they are mates, prey or settlement sites, is one of the most important challenges facing aquatic animals (e.g. Pawlik 1992). This is especially true for marine invertebrates that live in obligatory symbiotic interactions with hosts, and for which host location by larvae is critical for survival and successful growth. Invertebrate larvae rely on environmental cues—mainly soluble chemical metabolites released from the target organisms—for locating specific settlement sites such as prey (Pechenik *et al.* 1995) and conspecific adults (Toonen & Pawlik 1996). Waterborne chemical cues stimulate the larvae of some marine invertebrates to settle (Hadfield & Scheuer 1985; Zimmer-Faust & Tamburri 1994), the resulting behaviour commonly consisting of swimming arrest, which leads to a rapid descent in the water column, sometimes coupled with an increase in the swim turning rate (e.g. Tamburri *et al.* 1996). These behaviour patterns keep the larva as close as possible to the spot where it first encountered the cue, thus enhancing its chances of randomly discovering the target. Such a behavioural pattern may satisfy the settlement-site location needs of species inhabiting spacious substrates, e.g. algal turfs (Krug & Manzi 1999) or barnacle beds (Raimondi 1988), but is not effective for a larva that seeks spatially limited targets such as a specific small host animal. To cope with the problem of locating spatially limited settlement sites, it is more likely that the larva will have to actively follow the host's chemical plume upstream, against the flow, until it reaches the organism secreting

the odour. This orientation method relies on the fact that the target organism releases specific chemical metabolites which are carried downstream with the flow, forming a distinctive chemical plume that, depending on the magnitude of turbulent mixing, may resemble a trackable 'olfactory highway' (Nevitt *et al.* 1995).

Upstream tracking of chemical plumes is well known for species of crustaceans (Grasso & Basil 2002), but only for large adult animals; hitherto, plume tracking by invertebrate larvae has received little scientific attention. One of the few examples is of the cyprid larva of the parasitic barnacle *Heterosaccus dollfusi*, which may navigate upstream using chemotaxis to find its host, the crab *Charzybdis longicollis* (Pasternak *et al.* 2004). The aim of this present study was to substantiate the suggestion that specific, spatially limited settlement sites such as host organisms can be located in flow by diminutive marine larvae, using the combined cues of flow direction (rheo-orientation) and host chemicals (chemo-orientation). To address our aim, we quantitatively characterized the swimming pattern, direction, velocity, determination and turning rate, as well as host-location ability (HLA), of larvae of the symbiotic barnacle *Trevathana dentata* (formerly called *Savignium dentatum*) (Crustacea: Cirripedia). These factors were analysed in various combinations of flow and host metabolites, and their effects on the host-location behaviour of the larvae were examined. This study also revisited the findings of Brickner (1994), that *T. dentata* larvae become benthic and less mobile after the fourth naupliar instar. This would suggest that the host-locating phase is the third to fourth instar and not the cyprid, and our aim was to corroborate this phenomenon which has never been, to our knowledge, observed for any barnacle species.

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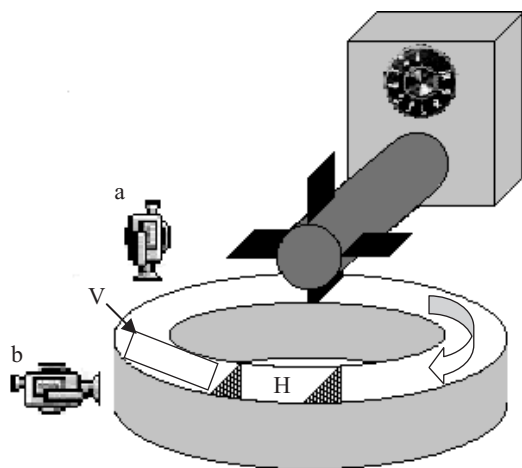


Figure 1. Flow-tank used to examine swimming patterns of *Trevathana dentata* larvae in various test solutions and flow conditions. A paddle wheel was used to generate a quasi-laminar flow which passed over the host cage (H) between two nylon mesh nets, into the camera-view field (V) where the larvae were released.

2. MATERIAL AND METHODS

(a) *Experimental set-up*

Larvae were tested under the following four combinations of flow and host metabolites: (i) still water without host odour; (ii) still water with host odour: chemical metabolites produced by the host while caged behind a permeable mesh inside the flume (figure 1, H); (iii) flow without host odour; and (iv) flow with host odour: in which larvae were released downstream from the host. Fluorescein-dye observations performed in still water before the actual experiments revealed that after *ca.* 1 h from the insertion of the host into the nylon mesh (pore size 70 μm) cage, the dye reached the farthest edge of the viewing field (*ca.* 30 mm; see figure 1, V); therefore, in all the experiments containing a host (in still water and flow), larvae were gently pipetted into the viewing field 1 h after insertion of the host into the mesh cage. Larval motion was then videotaped for 10 min using high-magnification lenses at 1–3 cm above the flume bottom. Contrary to most larval habitat selection experiments in flow, larvae were not allowed to pass over the host, but were only filmed in the viewing field downstream of the host, sensing only those host metabolites that had drifted with the flow. After 10 min of videotaping, the flume was emptied, washed and filled with fresh filtered seawater (FSW), and the experiment begun again using a fresh larval cohort and a new host colony. Each experimental combination was repeated three times, recording 10 individual larvae each time for a total of 30 larval trajectories per combination.

(b) *Flume*

The experiments were conducted in a transparent round Plexiglas flume (external diameter 30 cm, channel width 7 cm, height 5 cm; figure 1), in which 0.45 μm of FSW were circulated by a paddle wheel to a flow velocity of 5 mm s^{-1} . Annular flumes have been used in sediment-transport studies (Wainright 1990) and larval-motion studies (Hedvall *et al.* 1998), and are effective low-cost solutions for flow experiments. We examined the water-velocity profile using videofilm of neutrally buoyant passive particles (Pliolite VT, Goodyear Co., USA) in *ca.* 2 cm of water depth over the Plexiglas bottom at a velocity of 5 mm s^{-1}

in the centre part of the flume. Twenty particles were filmed at the horizontal plane, i.e. from above (figure 1, camera a) and another 20 at the vertical plane, i.e. from the side of the flume (figure 1, camera b). Motion trajectories for the particles were drawn and their direction of motion was analysed as explained below. The still water was analysed using the same methodology. It should be stressed that this study did not attempt to either fully describe the flow, or to simulate natural flow patterns over the seabed, but merely studied the behaviour and physical capabilities of the larvae in still and flowing water.

(c) *Animals*

Specimens of the reef coral *Cyphastrea chalcidicum* bearing its obligatory symbiont, the pyrgomatid barnacle *T. dentata*, were collected off the coast of Eilat, Israel. Barnacle shells were broken using a needle and the embryos within were pipetted and kept in tissue-culture dishes filled with FSW at room temperature until hatching. Larvae of *T. dentata* were found to be highly active as first to third instar nauplii, and at the second and third instars, exhibited high degrees of attraction to their host coral (i.e. most nauplii swam towards and then stayed upon a host coral introduced into their water). Both activity level and host attraction decreased from the fourth instar on, including the cyprid stage, and the animals continued their larval development while stationary on the ground or on their host. Hatched nauplii were transferred to 100 ml cups, and only 3–4-day-old nauplii were used in all experiments, because at this age the larvae exhibited the greatest level of activity and attraction to hosts. The host corals used in attraction experiments were checked carefully under a stereo-microscope to ensure that their bodies bore no barnacles or encrusting organisms of any kind; this was done to guarantee that experiments would not be biased by animals other than the host coral releasing chemical substances into the water.

(d) *Trajectory analysis*

Larval paths were observed only by camera a, for as long as they remained within the camera viewing field. Trajectories were manually drawn on acetate sheets at a sampling rate of one point per frame (25 points s^{-1}), and were subsequently digitized at a 1 point per pixel resolution using IMAGEJ, a public-domain image-processing software (see <http://rsb.info.nih.gov/ij>). Digitized trajectories were analysed using MATLAB, v. 6.5 (The MathWorks Inc.) to discover their direction of motion at each point in time. These data were then pooled for all the trajectories in the same experimental combination and presented as polar-axis histograms (figure 3). Also shown in figure 3 are the combined percentages of motion direction when the horizontal space is divided into the four 'compass directions', namely north (90°), south (270°), east (0°) and west (180°), when the host (if present) is located to the east and flow (if present) runs from east to west. We performed a Rayleigh test for each experimental combination to discover whether the circular distribution of swimming angles is uniform or directional: the length of the mean vector, r , was calculated ($r = 0$ for totally uniform and $r = 1$ for totally directed distribution) and its significance level was determined. MATLAB was also used to calculate the following characteristics for each trajectory: (i) swimming velocity; (ii) determination: the net displacement of the path, i.e. the distance between the first and last points, divided by the total path length and multiplied by 100; and (iii) rate of change of direction (RCDi): i.e. the total amount of degrees that the larva has turned divided by the path duration. For all trajectory characteristics,

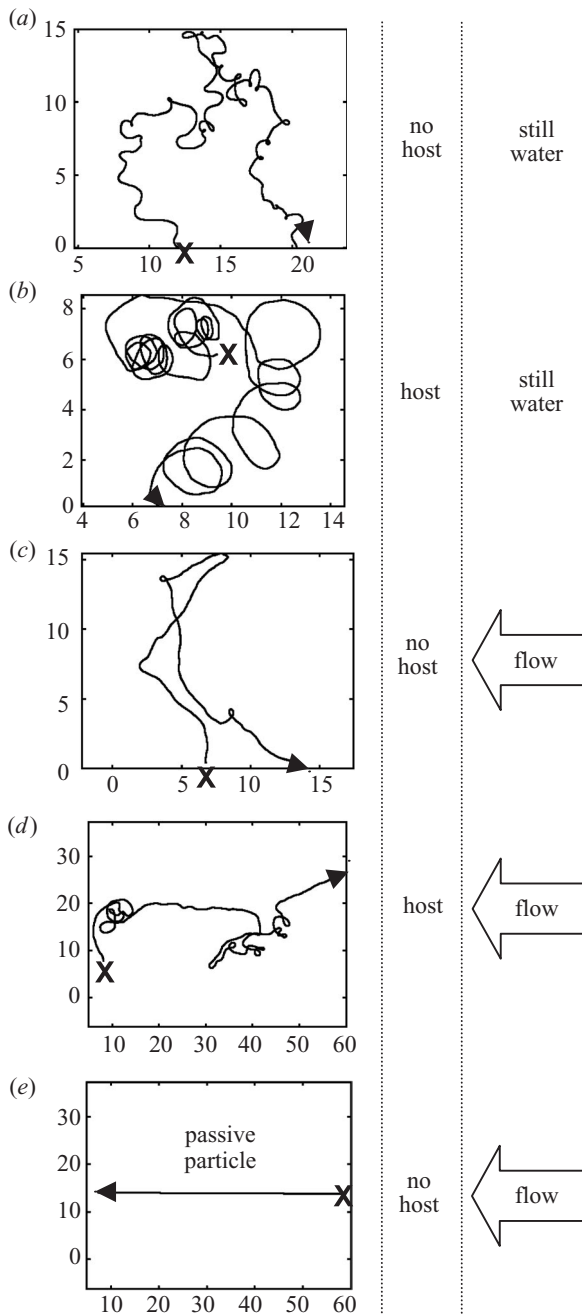


Figure 2. Typical examples of larval motion patterns. Typical motion patterns comprised at least 50% of motion patterns. The right column indicates flow condition and direction, the middle column indicates presence or absence of a host through which water passes *en route* to the larvae. The left column indicates the typical observed larval motion, where motion trajectories begin at the **X** symbol and end with a small arrow. Horizontal and vertical axes are in millimetres.

one-way ANOVA tests ($\alpha = 0.05$) were used to determine any possible differences, and subsequent *post hoc* Scheffe tests (at the 95% confidence interval) were used to determine the sources of these differences.

(e) Host-location ability

In addition to the above-mentioned experiments, another 20 larvae were randomly chosen in each of the experimental combinations and watched until they disappeared from the viewing

field or reached the nylon mesh. HLA was calculated as the percentage of larvae to reach the mesh, and this was repeated five times for a total of 100 larvae per combination.

3. RESULTS

Direction-analysis results of the passive particles from the vertical and horizontal planes did not vary significantly (one-way ANOVA test: $F = 0.20$, $p = 0.89$) and were therefore pooled. The particles exhibited a downstream motion that was almost totally straight (figures 2e and 3e; $r = 1.00$, $p < 0.01$), meaning that the flow had properties approaching laminar (i.e. 'quasi-laminar') in both planes. In still water, in the vertical plane, out of the 20 passive particles, four remained in the water column, nine sank to the bottom and seven rose to the surface, indicating some degree of motion in the water; however, this degree was judged to be small because in the horizontal plane, no significant motion of the particles could be detected.

In still water in the absence of odour, larvae exhibited a swimming pattern comprising mainly helical and circular motions (figure 2a). Swimming was uniformly directed (figure 3a; $r = 0.08$, $p > 0.05$), i.e. the larvae did not swim significantly in one direction over the others. In still water with host metabolites, larvae exhibited a swimming pattern consisting mainly of circular motion, with circle radii of *ca.* 1 mm (figure 2b). The direction of motion was again uniform (figure 3b; $r = 0.07$, $p > 0.05$). In flow without host metabolites, the larval behaviour consisted mainly of straight swim segments (figure 2c) directed perpendicularly to the flow, i.e. to the north and south (figure 3c). Because the Rayleigh test is designed to detect unidirectional biases, we performed one test for each hemisphere; in both north and south, swimming was found to be non-uniform ($r = 0.44$ and $r = 0.53$, respectively, $p < 0.05$). In flow with host metabolites, the larval motion consisted of two modes (figure 2d): straight motion, directed exclusively upstream and circular motion, directed mainly perpendicular to the flow. These two swimming modes alternated in time, and when combined, motion was found to be significantly directed upstream, towards the host (figure 3d; $r = 0.40$, $p < 0.05$).

Larval success in locating the host was $59.2 \pm 18.0\%$ when in flow with odour, significantly more than in all other treatments (figure 4; d.f. = 3, $F = 26.61$, $p < 0.01$). The swimming velocity of the larvae did not vary significantly between the different conditions (figure 4; d.f. = 3, $F = 2.37$, $p = 0.12$). When pooled, the swimming velocity was $2.8 \pm 1.8 \text{ mm s}^{-1}$. The swimming velocities we refer to in this study are the net velocities seen through the camera, and do not take into account the velocities needed to oppose the water flow; for example, a larva seen swimming directly upstream at 2.8 mm s^{-1} against a 5 mm s^{-1} flow is actually swimming at 7.8 mm s^{-1} . RCDi values were significantly different when in flow with host odour compared with all other conditions (figure 4; table 1; d.f. = 3, $F = 7.14$, $p < 0.01$). When larvae had limited access to directional cues, i.e. flow only, odour only or none of these, values were high: over 300 deg s^{-1} , whereas in the presence of both cues, RCDi decreased dramatically to a third of that level, 120 deg s^{-1} . Determination levels were found to be significantly different between the various

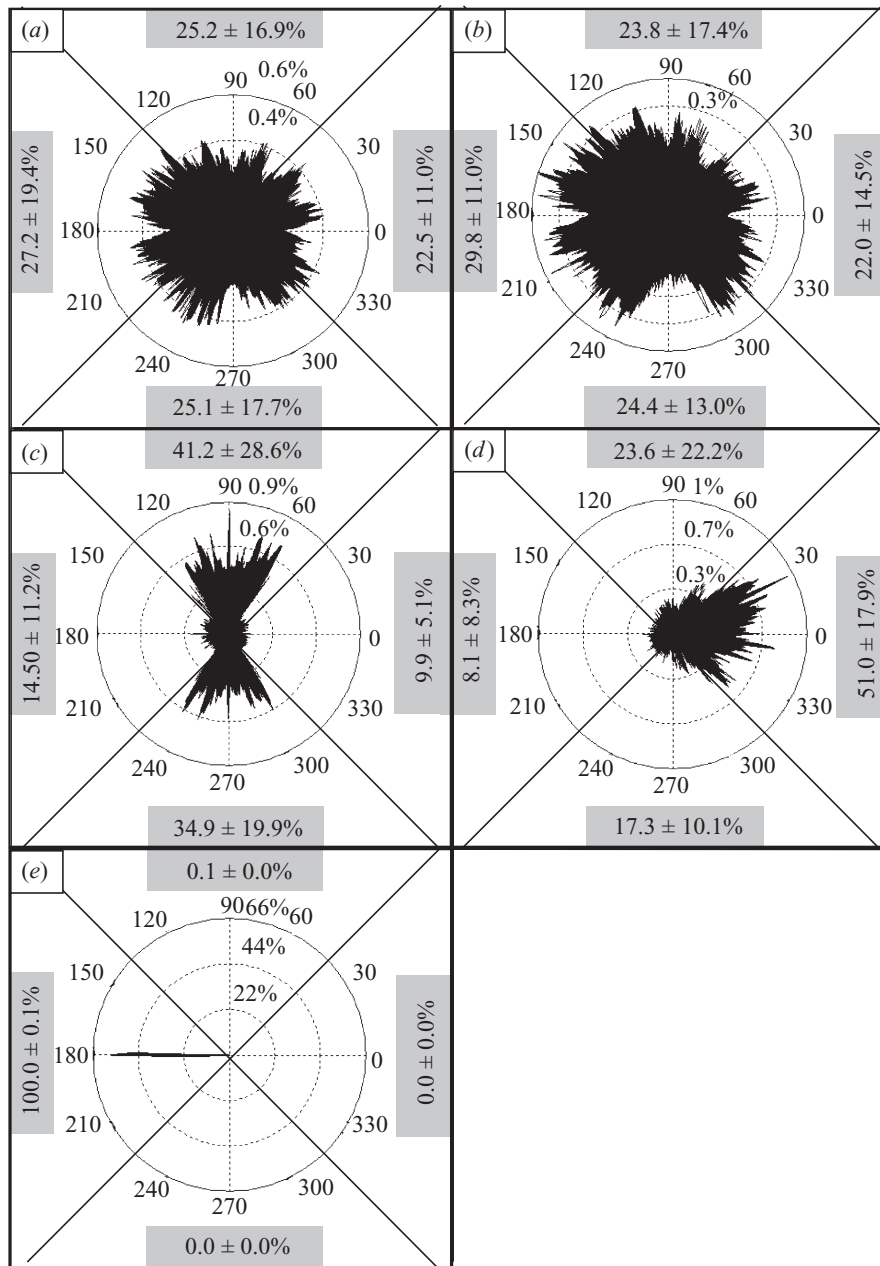


Figure 3. Directions of motion under the different experimental conditions: 0°, east; 180°, west; 90°, north; and 270°, south. The y-axis is a percentage of total motion. The numbers in grey panels are pooled percentages for each ‘compass direction’ (see text for further explanation). Circular x-axes, compass degrees; y-axes, direction of motion (%). (a) Still water without odour; (b) still water with odour; (c) flow without odour; (d) flow with odour; and (e) passive particles in flow.

Table 1. Summary of trajectory statistics in the different experimental conditions. Values (except for length of the mean vector, *r*) are mean ± 1 s.d.

condition		<i>n</i>	observation time (s)	length (mm)	velocity (mm s ⁻¹)	determination (%)	<i>r</i>	RCDi (deg s ⁻¹)	HLA (%)
flow	odour								
–	–	30	26.0 ± 14.4	81.8 ± 23.0	3.3 ± 1.1	8.7 ± 9.3	0.08	406.3 ± 195.8	2.9 ± 1.0
+	–	30	12.1 ± 7.4	29.6 ± 17.6	2.4 ± 1.5	66.4 ± 25.1	0.49	306.9 ± 88.5	4.7 ± 2.8
–	+	30	33.3 ± 29.8	96.5 ± 30.6	2.9 ± 0.7	6.6 ± 7.5	0.07	342.9 ± 111.0	1.1 ± 2.3
+	+	30	37.2 ± 24.2	83.5 ± 51.9	2.5 ± 0.9	58.6 ± 26.2	0.40	120.8 ± 92.2	59.2 ± 18.0
particles in flow		40	3.1 ± 0.3	15.4 ± 1.1	5.0 ± 0.5	99.7 ± 0.2	1.00	4.0 ± 3.0	0.0 ± 0.0

experimental conditions (figure 4; table 1; d.f. = 3, *F* = 12.01, *p* < 0.01). Subsequent *post hoc* Scheffe tests discovered two significantly different levels of determination:

a low level (under 20%) which occurred in the still-water treatments, and a high level (*ca.* 40–90%) which occurred in the flow treatments.

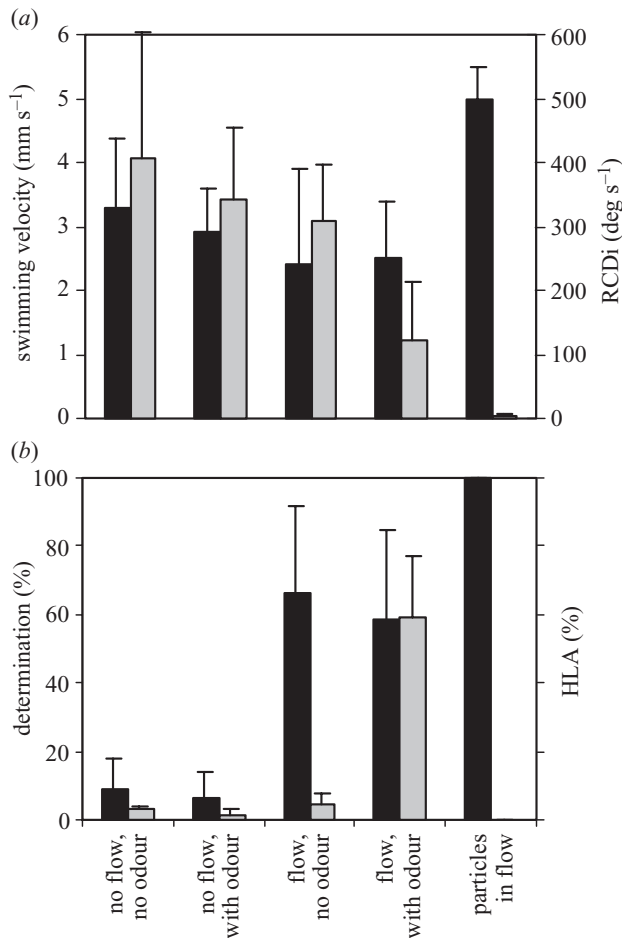


Figure 4. (a) Larval swimming velocity (black bars) and RCDi (grey bars) in the different experimental conditions. (b) Determination (black bars) and HLA (grey bars) in the different experimental conditions. Values are mean \pm 1 s.d.

4. DISCUSSION

Based on our findings, it is suggested that *T. dentata* larvae rely on both flow direction and host chemicals when searching for the host, and that they apply a motion model comparable to the odour-modulated anemotaxis of insects (e.g. David *et al.* 1983) and odour-gated rheotaxis of blue crabs (Weissburg & Zimmer-Faust 1994). The model scheme may be demonstrated in its entirety using figures 2d and 4: when perceived host-metabolite level rises above a threshold detectable value, the larva surges upstream in the odour plume, in a straight path (low RCDi) and with high determination. Once the larva ceases sensing the attractant, e.g. when exiting the plume or experiencing a concentrational drop within it, it commences a cross-flow casting pattern which has the same high determination but a more circular path. Upon re-sensing the chemical, the larva once again changes its swimming to a surge pattern, and so on, until it reaches the host in a series of cast-and-surge moves. Thus, the odour stimulation is involved in the onset and modulation of motion, while the direction of movement is determined according to the direction of the flow, either upstream (in surge phases) or cross-stream (in cast phases).

Although *T. dentata* larvae are capable of effective location of their host in flow using odour-gated rheotaxis, they are unable to use chemotaxis to perform the same

task in the absence of flow (figures 2b and 4). This observation may suggest that the larvae do not react to small spatial or temporal concentration changes of the chemical attractant (as would be required for chemotaxis), but rather only to its presence or absence (as is required for odour-gated rheotaxis). Adopting such a simple mechanism of behaviour may mean that the larvae can perform host location without a complex chemosensory array (see also Grasso & Basil 2002); such supposed simplification may preclude 'classical' chemotaxis but help reduce the time required for the larvae to achieve sufficient host-location capabilities.

The cyprid stage is considered to be the larval stage that locates the settlement substrate in all the cirripedes (Anderson 1994), and this research is the first, to our knowledge, to document substratum location performed potentially by the earlier naupliar stages. Although research on chemical sensing in cirripede nauplii is somewhat lacking, it has been shown that there are several sensory structures on the naupliar dorsal carapace that are supposedly involved in chemical sensing (Walker & Lee 1976), and that balanomorph nauplii may react to chemicals carried by the flow (Yule 1986). As for flow sensing, which is needed for rheotaxis, this may be performed by the frontal filament complex. These sensory organs comprise a vesicle and a filament, and although their function is not completely understood, Walker (1974) suggested that the vesicle reacts to changes in pressure while the filament senses the water flow.

Trevathana dentata nauplii require two weeks to develop into the cyprid stage, and in that time they can potentially be carried several kilometres by the flows of the Gulf of Eilat (Abelson *et al.* 1999), to settle on coral colonies far away from their 'parent' colonies. Despite this ability to reach remote sites, observations suggest that settled larvae can be found on the host coral colonies from the very first day of the reproduction season (Brickner 1994). These observations imply that settling larvae originate from nearby colonies, and that the larvae actually spend much less time in the water column, and travel much shorter distances than is expected. This phenomenon is well known from other organisms: for example, *Haliotis rubra* demonstrates a larval dispersal range that does not exceed several dozen metres, although the larvae may be in the water for several days (Prince *et al.* 1988) and have the potential of being distributed far larger distances. The findings of Brickner (1994) fit nicely with our discovery about the HLA of the nauplii, and with the observations that laboratory-reared nauplii decrease their activity level from the fourth instar on, including the cyprid stage, and finish their larval development while stationary. It may be concluded that the cypris stage of *T. dentata* is not likely to have a role in host location, but rather plays a sole role in the later process of physical attachment to the host, being able to make a fixed association with the host and subsequently metamorphose into an adult barnacle.

We thank M. Gur, S. Clodong, R. Olinky, S. Shefer, I. Brickner, O. Mokady and I. Golani. We also thank the Interuniversity Institute of Eilat for assistance and use of facilities. This research was funded by a grant from the Israel National Science Foundation of the Israel Academy of Sciences. This work is part of

a thesis of Z.P. towards his PhD degree. Z.P. was also supported by a grant from the Minerva foundation, Germany, and B.B. was supported by the VW Stiftung, Germany.

REFERENCES

- Abelson, A., Steinman, B., Fine, M. & Kaganovsky, S. 1999 Mass transport from pollution sources to remote coral reefs in Eilat (Gulf of Aqaba, Red Sea). *Mar. Poll. Bull.* **38**, 25–29.
- Anderson, D. T. 1994 *Barnacles: structure, function, development and evolution*. London: Chapman & Hall.
- Brickner, I. 1994 Ecology, systematics and phylogenesis of coral-inhabiting barnacles from the gulf of Eilat. PhD thesis, Bar-Ilan University, Ramat-Gan, Israel.
- David, C. T., Kennedy, J. S. & Ludlow, A. R. 1983 Finding of a sex pheromone source by gypsy moths released in the field. *Nature* **303**, 804–806.
- Grasso, F. W. & Basil, J. A. 2002 How lobsters, crayfishes and crabs locate sources of odor: current perspectives and future directions. *Curr. Opin. Neurobiol.* **12**, 721–727.
- Hadfield, M. G. & Scheuer, D. 1985 Evidence for a soluble metamorphic inducer in *Phestilla*: ecological, chemical and biological data. *Bull. Mar. Sci.* **37**, 556–566.
- Hedvall, O., Moksnes, P. E. & Pihl, L. 1998 Active habitat selection by megalopae and juvenile shore crabs *Carcinus maenas*: a laboratory study in an annular flume. *Hydrobiol.* **375/376**, 89–100.
- Krug, P. J. & 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.
- Nevitt, G. A., Veit, R. R. & Karieva, P. M. 1995 Dimethyl sulfide as a foraging cue for antarctic Procellariiform seabirds. *Nature* **376**, 680–682.
- Pasternak, Z., Blasius, B. & Abelson, A. 2004 Host location by larvae of a parasitic barnacle: larval chemotaxis and plume tracking in flow. *J. Plankt. Res.* **26**, 487–493.
- Pawlik, J. R. 1992 Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. A. Rev.* **30**, 273–335.
- Pechenik, J. A., Hadfield, M. J. & Eyster, L. S. 1995 Assessing whether larvae of the opisthobranch gastropod *Phestilla sibogae* Bergh become responsive to three chemical cues at the same age. *J. Exp. Mar. Biol. Ecol.* **191**, 1–17.
- Prince, J. D., Sellers, T. L., Ford, W. B. & Talbot, S. R. 1988 Confirmation of a relationship between the localized abundance of breeding stock and recruitment for *Haliotis rubra* Leach (Mollusca: Gastropoda). *J. Exp. Mar. Biol. Ecol.* **122**, 91–104.
- Raimondi, P. T. 1988 Settlement cues and determination of the vertical limit of an intertidal barnacle. *Ecology* **69**, 400–407.
- Tamburri, M. N., Finelli, C. M., Wethey, D. S. & Zimmer-Faust, R. K. 1996 Chemical induction of larval settlement behavior in flow. *Biol. Bull.* **191**, 367–373.
- Toonen, R. J. & Pawlik, J. R. 1996 Chemical induction of larval settlement behavior in flow. *Mar. Biol.* **126**, 725–733.
- Wainright, S. C. 1990 Sediment-to-water fluxes of particulate material and microbes by resuspension and their contribution to the planktonic food web. *Mar. Ecol. Prog. Ser.* **62**, 271–281.
- Walker, G. 1974 Fine-structure of frontal filament complex of barnacle larvae (Crustacea-Cirripedia). *Cell Tissue Res.* **152**, 449–465.
- Walker, G. & Lee, V. E. 1976 Surface structures and sense organs of the cypris larva of *Balanus balanoides* as seen by scanning and transmission electron microscopy. *J. Zool. Lond.* **178**, 161–172.
- Weissburg, M. J. & Zimmer-Faust, R. K. 1994 Odor plumes and how blue crabs use them in finding prey. *J. Exp. Biol.* **197**, 349–375.
- Yule, A. B. 1986 Changes in the limb beat movements of barnacle nauplii in the presence of food organisms. *J. Exp. Mar. Biol. Ecol.* **103**, 119–129.
- Zimmer-Faust, R. K. & Tamburri, M. N. 1994 Chemical identity and ecological implications of a waterborne, larval settlement cue. *Limnol. Oceanogr.* **39**, 1075–1087.