J. exp. Biol. **137**, 141–156 (1988)
Printed in Great Britain © *The Company of Biologists Limited 1988*

NEGATIVE GEOTAXIS IN SEA URCHIN LARVAE: A POSSIBLE ROLE OF MECHANORECEPTION IN THE LATE STAGES OF DEVELOPMENT

BY YOSHIHIRO MOGAMI, CHIEKO OOBAYASHI*, TOMOKO YAMAGUCHI†, YUMI OGISO‡ AND SHOJI A. BABA

Department of Biology, Ochanomizu University, Otsuka 2-1-1, Tokyo 112, Japan

Accepted 25 January 1988

Summary

Negative geotactic behaviour of sea urchin larvae at various developmental stages from blastula to pluteus was analysed by means of time-exposure dark-field photography of the swimming behaviour of individual larvae. Significant differences in the patterns of behaviour, such as swimming direction and speed, were demonstrated between the early stages (up to the gastrula) and the pluteus, although larvae at any developmental stage showed negative geotactic migration. Larvae in the early stages swam at speeds that varied as a function of the swimming direction with respect to gravity, faster downwards and slower upwards. This might be predicted from the assumption that vertical locomotion is determined by constant propulsion affected passively by gravity. In the pluteus stage, however, larvae swam at a constant speed in any direction, suggesting that the propulsive activity of swimming plutei is actively controlled depending on the swimming direction. This change in the negative geotactic behaviour of sea urchin larvae in the course of embryogenesis indicates development of physiological control systems for propulsive activity at the pluteus stage.

Introduction

Vertical positioning in a water column is thought to be important for planktonic marine invertebrate larvae in introducing themselves into a suitable environment, especially for food gathering. Since the specific gravity of the larvae is usually greater than that of sea water, they cannot remain suspended at a given vertical position, but must overcome the effects of gravity. Numerous devices have been

* Present address: Dinabot, Matsudo, Chiba.
† Present address: Mitsubishi Chemical Industries, Research Center, Yokohama, Kanagawa.
‡ Present address: Denenchofu Futaba Gakuen, Setagaya, Tokyo.

Key words: geotaxis, sea urchin, larvae.

developed by marine invertebrate larvae to overcome passive sinking due to gravity (for a review, see Chia, Buckland-Nicks & Young, 1983). Migration against the acceleration of gravity, i.e. negative geotaxis, is considered to be one of the mechanisms behind the uneven distribution of larvae in a water column, and is thought to be essential for the larvae of benthic organisms moving towards the surface from the sea bottom where they are liberated from the adults.

Mechanisms for geotactic orientation and locomotion have been studied intensively in unicellular, ciliated protozoa. Two basic mechanisms are summarized here; (a) physical mechanisms where geotactic behaviour is based purely on the mechanical properties of the organisms, non-uniformity of density, differential drag on body regions or uneven locomotor activity which generates gyration, and (b) physiological mechanisms whereby locomotor organelles are regulated differentially by varying orientation which is sensed by some 'functional statocyst' (for a review, see Bean, 1984). Recent studies which obtained quantitative measurements of swimming vectors compared with static equilibrated orientation distribution and swimming pathways in *Paramecium* (Fukui & Asai, 1980, 1985) have supported a purely physical mechanism based on gravity-buoyancy orientation, originally proposed by M. Verworn (cited by Fukui & Asai, 1985). Physiological mechanisms including gravireception, however, have been demonstrated in the ciliated protozoon *Loxodes* which has Müller vesicles, a statocyst-like organelle (Fenchel & Finlay, 1986). *Loxodes* alters the sign of geotaxis depending on environmental dissolved oxygen tension, almost always responding to the experimental inversion of its body by turning to resume its previous orientation (Fenchel & Finlay, 1984).

Physical mechanisms proposed for negative geotaxis in ciliated protozoa would also be applicable, if they were correct, to planktonic marine invertebrate larvae because both organisms are subject to low Reynolds number conditions. Geotaxis in marine invertebrate larvae may depend largely on physiological mechanisms, at least in those with statocysts, because their multicellular organization could equip them with specific sensory organs. Because of the changes in shape and size of the body and arrangement of locomotor organs during morphogenesis, the mechanisms larvae use for geotaxis may differ at different stages. Therefore, studies using marine invertebrate larvae may help to explain the mechanisms of geotaxis.

To examine mechanisms of geotaxis in multicellular micro-organisms we examined the negative geotactic behaviour of larvae of the sea urchins *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*, ciliated micro-organisms which undergo rapid morphological changes accompanied by organogenesis. Special attention was paid to changes in behaviour in the course of embryogenesis, from blastula with the simplest cellular organization to pluteus with several specific organs. In this study we measured the short-term movement of individual larvae with respect to gravity, and report quantitative as well as qualitative changes during development in negative geotactic behaviour by analysing the vector velocities of swimming. The results of the analyses indicate that geotactic movements of plutei may be actively controlled by some physiological systems first

developed at the pluteus stage. Larvae in earlier stages may migrate geotactically, depending mostly on their own mechanical properties.

Materials and methods

Sea urchin larvae (*Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*) were grown in Jamarin U artificial sea water (ASW) (Jamarin Laboratories, Osaka) following the procedures used by Degawa, Mogami & Baba (1986). After hatching, swimming larvae at various developmental stages (12–60 h after insemination, corresponding to blastula to pluteus) were collected by hand centrifuge and washed several times with ASW containing (in mmol l^{-1}): NaCl, 450; KCl, 10; $CaCl_2$, 10; $MgSO_4$, 28; $MgCl_2$, 25; Tris-HCl, 10; pH 8·0. Larvae suspended in ASW at densities of $1–6\times10^2$ larvae ml^{-1} were then transferred into air-bubble-free chambers 0·5 mm × 20 mm × 20 mm made up of a slide, cover and frame-shaped silicon rubber spacer of 0·5 mm thickness. The thin water layer in the chamber limited free swimming of larvae virtually to the plane parallel to the slide and cover, which were placed perpendicular (horizontal arrangement) or parallel to gravity (vertical arrangement).

The swimming behaviour of larvae in the chambers was recorded by time-exposure dark-field photography. The exposure period was usually 8·2 s. During recordings of the vertical arrangement, the first 0·6 s was lit more brightly than the rest to distinguish the start of the recordings. The intensity and direction of illumination of the experimental chamber were fixed throughout the experiments. The illumination was designed in the vertical arrangement to illuminate the chamber at right angles to gravity and oblique to the wider chamber walls. The horizontal arrangement followed that of Degawa *et al.* (1986), except that a circular fluorescent lamp was used for continuous lighting. Recording equipment was shaded by hoods from direct room light. During experiments the larvae were left for at least 10 min before the start of recording to minimize the effects of mechanical disturbance by transfer of larvae into the chamber. After recordings had been made in the horizontal arrangement, the chamber was lifted at one end to bring it into the vertical arrangement (V-rearrangement) in which recordings were made immediately and after 30 s. After more than 10 min in this arrangement, the chamber was inverted and the recording sequence repeated (V̄-rearrangement). Recordings were made at room temperature, 18–22 °C for *Hemicentrotus* and 22–24 °C for *Anthocidaris*.

The swimming paths recorded under dark-field illumination were analysed for individual larvae by minicomputer (Okitac 50/10, Oki Electric, Tokyo) interfaced with a digitizer (Gradimate U4-30, Oscon Co., Tokyo), on which the paths were processed point by point from printed photographs enlarged approximately 7–15 times. For analysis of swimming paths, those interrupted by the edge of the chamber were rejected. Swimming velocities of individual larvae were calculated from the lengths of paths recorded during the fixed exposure time. These were calculated as the sum of the distances between successive points taken along the

paths. Swimming direction relative to gravity was calculated from the coordinates at the beginning and end of the paths.

Results

Negative geotactic behaviour of sea urchin larvae

Typical recordings of geotactic migration of sea urchin larvae are shown in Fig. 1. Swimming occurred randomly in all directions parallel to the wider walls of the chamber which were placed perpendicular to gravity (horizontal arrangement) (Fig. 1A). The larvae began to swim upwards immediately after the chamber had been changed to the vertical arrangement (Fig. 1B). Upward migration continued until it was interrupted by the edge of the silicon rubber spacer, resulting in a dense accumulation of larvae at the top of the chamber (Fig. 1C). Pattern swimming (or bioconvection), often reported from other organisms capable of negative geotaxis, did not occur under our experimental conditions. However, it was often observed when the population density of larvae was increased to 2×10^4 larvae ml^{-1} (Yasumasu, 1963), or if the distance between the slide and cover was extended to 3 mm using thicker silicon rubber spacers. When the chamber with the larvae accumulated at its top was inverted ($\bar{V}$-rearrangement), larvae began to migrate upwards again (Fig. 1E,F).

Both negative geotactic behaviour and ciliary beating of the larvae ceased when up to 1 mmol l^{-1} KCN was added to the medium.

As shown in Fig. 1, negative geotactic migration occurred as soon as the chamber was brought into the vertical arrangement. Recordings of 8·2-s exposures immediately after V- and $\bar{V}$-rearrangements showed many curved swimming paths, consistent with negative geotactic reorientation of individuals (Fig. 1B,E). Swimming paths became straighter, and upwards, in the vertical arrangement, suggesting strong negative geotaxis, as demonstrated in recordings made 30 s after the rearrangements (Fig. 1C,F). The geotactic behaviour was analysed further from the recordings at 30 s after both types of rearrangements (Fig. 1C,F).

Developmental changes in negative geotactic behaviour

Sea urchin larvae at various developmental stages showed negative geotactic behaviour. Although most of the population migrated upwards and finally accumulated at the top of the chamber, the swimming behaviour of individuals changed during development. As shown in Fig. 2, remarkable changes, especially in the swimming direction, occurred after prism stage. Larvae at blastula and gastrula stages swam preferentially upwards with straight swimming paths almost parallel to gravity (Fig. 2A,B). Larvae after prism stage also swam primarily upwards but a substantial number of them swam along comparatively straight paths in other directions, apparently independent of gravity (Fig. 2C,D).

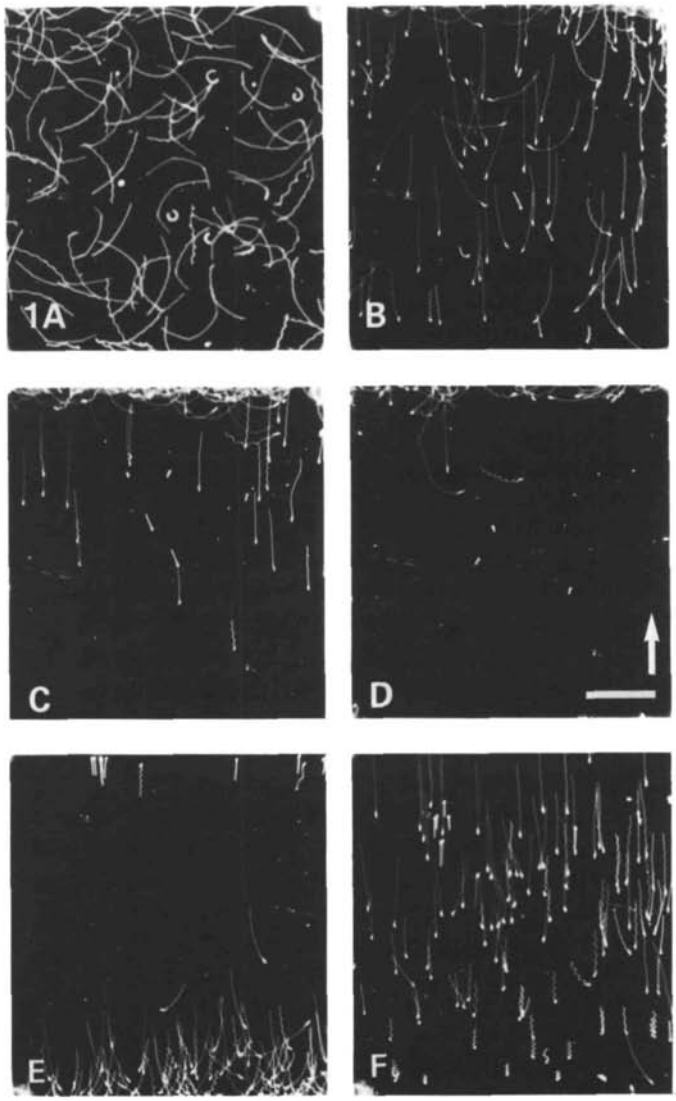

Fig. 1. Dark-field recordings of negative geotactic migration of sea urchin (*Anthocidaris crassispina*) larvae at the blastula stage (12 h after insemination). The chamber containing larvae was placed either horizontally (A) or vertically (B–F). Recordings were at 0 min (B), 0·5 min (C) and 10 min (D) after the chamber had been set vertically from the horizontal arrangement (V-rearrangement), and at 0 min (E) and 0·5 min (F) after inversion of the chamber (V̄-rearrangement). Exposure time was 8·2 s and the first 0·6 s was lit more brightly in B–F. The arrow indicates the upward direction. Scale bar, 5 mm.

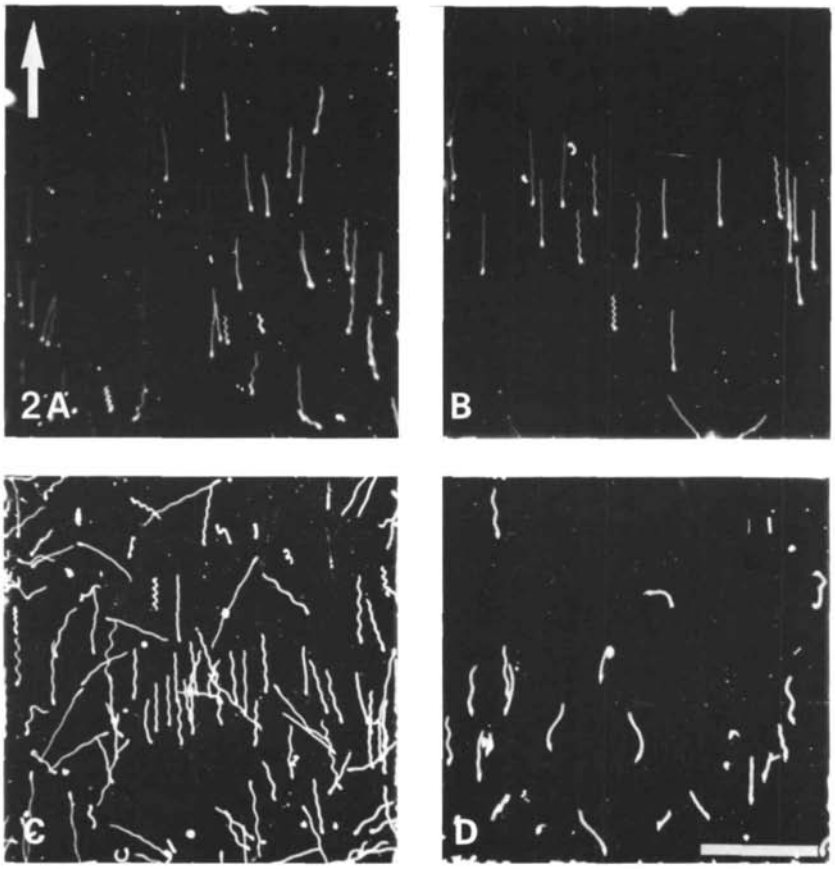

Fig. 2. Dark-field recordings of negative geotactic migration of sea urchin (*Hemicentrotus pulcherrimus*) larvae at the blastula stage 16 h after insemination (A), gastrula at 28 h (B), prism at 37 h (C) and pluteus at 55 h (D). Swimming paths were recorded in the V-rearrangement. The arrow indicates the upward direction. Exposure, 8·2 s, with the first 0·6 s at brighter illumination. Scale bar, 5 mm.

The swimming vector defined by the start and end of the swimming path of individual larvae recorded in 8·2-s exposures was analysed to characterize larval swimming velocity and direction at different developmental stages. In Fig. 3 vectors are plotted as dots representing the leading end of vectors starting from the origin. Strong differences were noted between gastrula and prism stages. In the early stages most of the dots were concentrated around the ordinates on the positive side, indicating that most of the larvae swam preferentially upwards (Fig. 3A,B). In the later stages (post prism) the dots were scattered broadly, indicating that the larvae did not swim only upwards (Fig. 3C,D), although in the vertical arrangement (not shown) most of the population finally accumulated at the upper edge of the chamber after approximately 10 min.

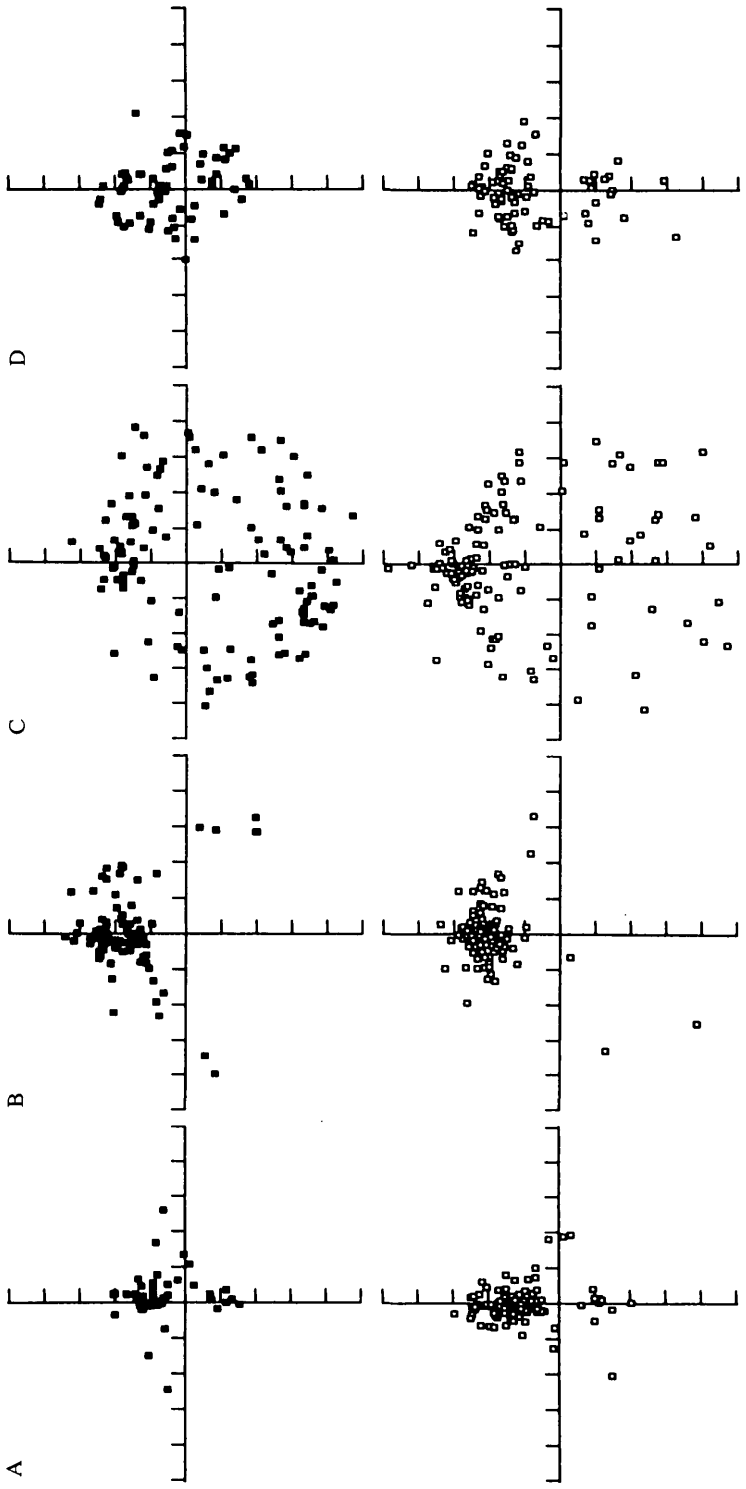

Fig. 3. Swimming vectors obtained from the swimming paths of individual larvae of *Hemicentrotus pulcherrimus*. The points indicate the leading end of vectors originating from the origin. The upward direction is at the top of the figure. Upper and lower halves of the figure are for vectors obtained in the V- (filled symbols) and V̄-rearrangements (open symbols), respectively. The vectors of blastula 16 h (A) after insemination, gastrula 22 h (B), prism 37 h (C) and pluteus 55 h (D) stages are shown. Scale divisions, 0·1 mm s^{-1}.

For further analysis of swimming directions the average of normalized swimming vectors was calculated as:

$$\mathbf{V} = (1/n) \sum^{n} \mathbf{v}/v ,$$

where $\mathbf{v}$ and v are the swimming vector and its magnitude obtained from each larva, and n is the number of larvae analysed for each developmental stage in a series of experiments. The averaged vector $\mathbf{V}$ was a measure of the average migration of the larval population; the direction and magnitude of the vector represent the direction of migration of the population and the degree of scattering of the swimming directions among the individuals. Fig. 4 shows values of $\mathbf{V}$ from larvae in the V- and $\bar{V}$-rearrangements at the different developmental stages. If all the larvae migrated in the same direction, $\mathbf{V}$ would equal unity, so that $\mathbf{V}$ would be plotted on the circumference of a circle with a radius of unity when represented by dots as in Fig. 3. However, the more randomly the larvae swim, the nearer the vectors would be to the origin. The concentration of dots around the upper half of the ordinate in Fig. 4 indicates that at all stages the direction of migration was upwards. Fig. 4 also indicates that the vertical component of upward migration was larger prior to prism stage; i.e. the larvae tended to swim more randomly after prism stage, as shown in Figs 2, 3. The upward migration appeared to be enhanced in the $\bar{V}$-rearrangement (Fig. 4B) compared with that in the V-rearrangement (Fig. 4A). Points for the $\bar{V}$-rearrangement were more concentrated in the upper region of the ordinate. It should be noted that the enhancement was more apparent in the later stages than in the earlier ones, although the characteristic differences between the developmental stages were maintained.

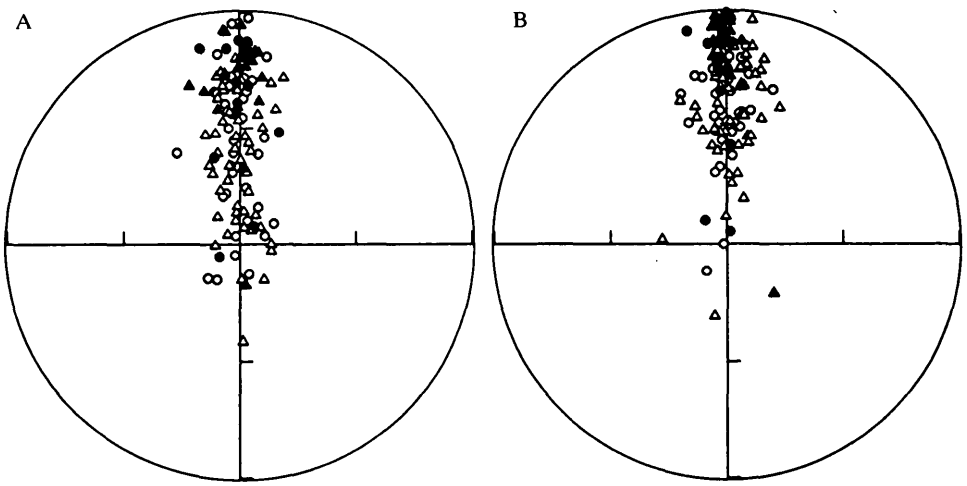

Fig. 4. Normalized and averaged swimming vectors $\mathbf{V}$ obtained from the larvae of *Hemicentrotus pulcherrimus* in the V-rearrangement (A) and $\bar{V}$-rearrangement (B). The symbols indicate the leading end of $\mathbf{V}$ (●, blastula; ▲, gastrula; ○, prism; △, pluteus) originating from the origin. Upwards is the top of the figure. Each symbol represents a value from 20–150 larvae. For calculation of $\mathbf{V}$, see text.

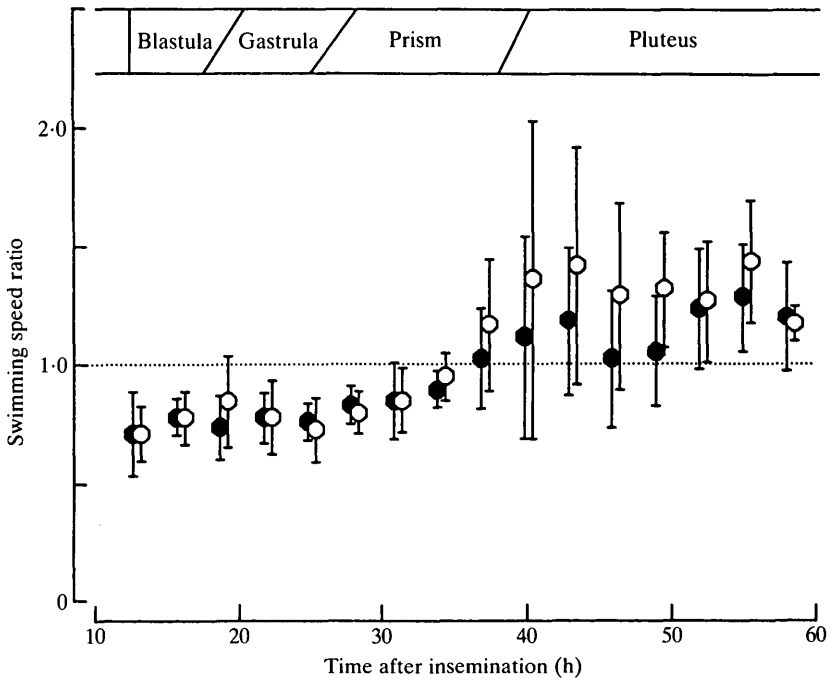

Fig. 5. Ratio of swimming activity, vertical: horizontal, of *Hemicentrotus pulcherrimus* as a function of time after insemination. The ratios of the mean swimming velocities in the V-rearrangement (filled symbols) and V̄-rearrangement (open symbols) *vs* horizontal swimming are averaged within each time range at 3-h intervals, from 10 h after insemination. Each symbol with an S.D. bar represents the mean of the ratios obtained from 7–15 separate measurements (20–150 larvae per measurement). Developmental stages based on morphology are indicated at the top of the figure.

The mechanical conditions differed in the horizontal and vertical arrangements of the chamber. In the horizontal one the larvae were kept from sinking by the wall of the chamber. However, in the vertical one their swimming velocity would be reduced when swimming upwards, and increased when swimming downwards, because of the additive effect of sinking. The average swimming velocity measured in the vertical arrangement should therefore be smaller than that measured in the horizontal arrangement if most of the population swims preferentially upwards in the vertical arrangement. If this were the case, the ratio of the average swimming velocity, vertical to horizontal, should increase with a decreasing population of larvae swimming upwards. Higher values could therefore be anticipated for prism and pluteus stages, when the larvae tend to swim in more random directions, compared with those in the early stages. The observed ratios were smaller than unity in the early stages and exceeded unity in the later stages (Fig. 5). This is consistent with the assumption above.

The influence of angular direction on swimming velocity was analysed from direct measurements of the swimming vectors of individuals. If the assumption of

the additive effect of sinking is valid in the swimming behaviour of sea urchin larvae, velocity should change according to the swimming direction following a simple equation derived from vector calculus. The Reynolds number, *Re*, based on the larval radius, r, was defined as:

$$Re = rU\rho_o/\eta ,$$

where U, ρ_o and η are the speed, density and viscosity of the fluid, respectively. *Re* was estimated to be 0·01–0·03 by taking $r = 70–80\,\mu m$, $U = 0·1–0·4\,mm\,s^{-1}$, $\rho_o = 1·025\,g\,cm^{-3}$ and $\eta = 10^{-4}\,Pa \cdot s$ (the former two were measured directly and the latter two were for ASW at 20°C). Since the vector velocities of the fluid are approximately additive at such low Reynolds numbers (Happel & Brenner, 1973), the equation

$$p^2 = v^2 + s^2 - 2vs\cos\theta$$

and therefore

$$v = \sqrt{(p^2 - s^2\sin^2\theta)} + s\cos\theta$$

can be derived by vector calculus for the swimming direction θ from 0° (downwards) to 180° (upwards) (Roberts, 1970), where v, p and s are the resultant velocity as a function of θ, and the magnitude of propulsive and sinking velocities, respectively. If there were no alteration of propulsive activity (p remained unchanged), and if the velocity of sinking did not change irrespective of the orientation of larvae, the theoretical curve of v *vs* θ is sigmoid, as shown in Fig. 6A,C. In these figures p was assumed to be equal to the average swimming velocity measured in the horizontal arrangement, and s was the average falling speed of immobilized larvae in the presence of $1\,mmol\,l^{-1}$ KCN. The values of v *vs* θ observed from larvae at gastrula or earlier stages closely agreed with the theoretical values shown in Fig. 6A, although they were smaller at low θ (0–50°) than those predicted from the equation above. This was probably due to the contribution of unusually inactive larvae to this range. The agreement in the range of $\theta > 50°$ was statistically significant ($P < 0·05$, Spearman rank correlation). However, the values from plutei completely violated the theoretical curve, demonstrating a remarkable constancy of swimming speed over the whole range of θ (Fig. 6C), suggesting some controlled alteration of p. It is obvious that the observed values of v for larvae at gastrula and earlier stages may be consistent with the theoretical values, and that the assumption of constancy in p is wrong for plutei. This suggests that there must be some mechanism, probably physiological, in the pluteus stage which adjusts propulsion, and has not been acquired in the early developmental stages.

Discussion

In this paper we have demonstrated that negative geotactic behaviour of sea urchin larvae varies according to developmental stage. In the early stages, at blastula and gastrula, larvae swam preferentially upwards with a reduction in velocity equal to the sinking rate. In the stages following prism, however, larvae

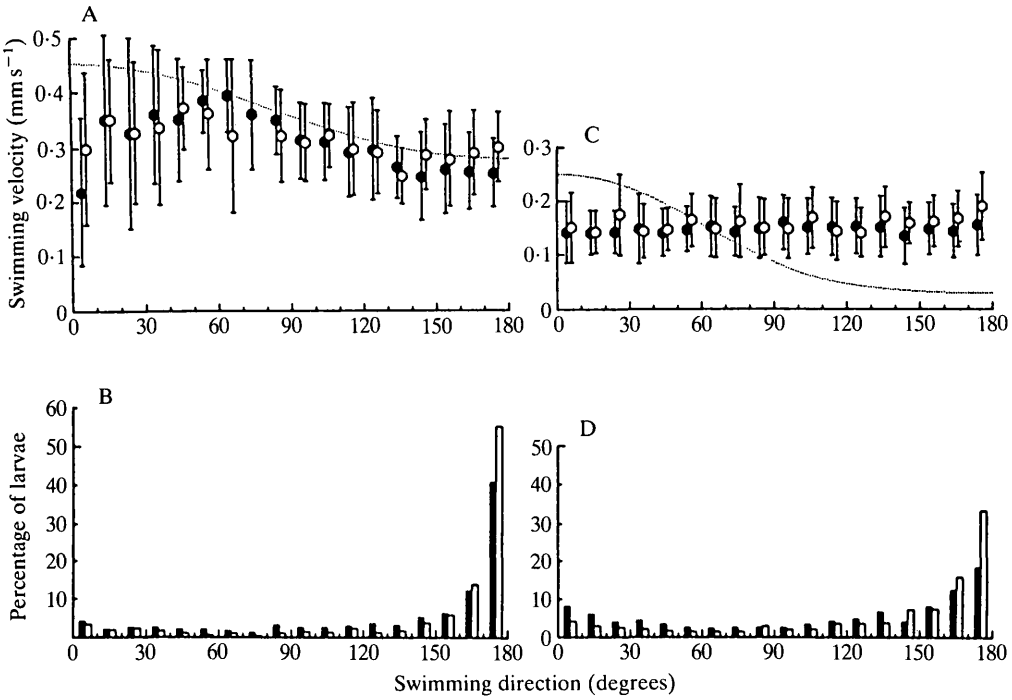

Fig. 6. Swimming velocity of larvae of *Hemicentrotus pulcherrimus* as a function of swimming directions. (A,C) The magnitude of velocity v *vs* the angular direction with respect to gravity of swimming in gastrula (19–28 h after insemination) (A) and pluteus (43–58 h) (C) stages. Values of v are averaged within each range of θ, at 10° intervals from 0° (downward) to 180° (upward), and represented by filled symbols for the V-rearrangement and open symbols for the $\tilde{V}$-rearrangement, both with S.D. bars. (B,D) The percentages of the larvae used in A and C, respectively, that swim in θ of the ranges above are shown by filled columns (V-rearrangement) and open columns ($\tilde{V}$-rearrangement). The total numbers of larvae were 749 (V-rearrangement) and 941 ($\tilde{V}$-rearrangement) in B and 697 (V-rearrangement) and 603 ($\tilde{V}$-rearrangement) in D. For the theoretical curves defined in the text (dotted lines in A and C), the velocity of propulsion, p, was assumed to be equal to the swimming velocity measured in the horizontal arrangement, and the sinking speed, s, was measured from immobilized larvae bathed in ASW containing 1 mmol l^{-1} KCN when falling in steady state. For gastrula stage, $p = 0{\cdot}365 \pm 0{\cdot}082$ mm s^{-1} (mean ± S.D.) ($N = 1389$) and $s = 0{\cdot}087 \pm 0{\cdot}019$ mm s^{-1} ($N = 739$). For pluteus stage, $p = 0{\cdot}140 \pm 0{\cdot}051$ mm s^{-1} ($N = 1334$) and $s = 0{\cdot}111 \pm 0{\cdot}026$ mm s^{-1} ($N = 870$).

tended to swim more randomly at a constant velocity, indicating actively controlled propulsion. This implies developmental changes in two parameters of swimming behaviour: larval orientation and swimming activity.

Preliminary measurements of the orientation of steadily falling immobilized larvae demonstrated that larvae in blastula and gastrula oriented preferentially upwards, whereas those in prism and pluteus tended to orient themselves randomly. The distribution of larval orientation (not shown) was consistent with

that of the swimming direction (Fig. 6B,D). This suggests that the orientation of larvae may be mostly mechanically determined because of asymmetrical distribution of body mass, as reported by Pennington & Emlet (1986). Changes in their mechanical properties during embryogenesis would result in variation of the orientation of freely swimming larvae in the later stages.

Support for active, physiological control of swimming activity in plutei comes from the observation that the theoretical curve derived from the assumption of the passive effect of gravity is not consistent with the swimming velocity distribution directly measured for individual plutei (Fig. 6C), although the curve almost fitted the distribution of swimming velocities measured in the gastrula stage (Fig. 6A). It may be profitable for further discussion to evaluate the effects on the theoretical curves of the variations in sinking speed, s, since strictly speaking s would vary depending on θ. The sinking speed of a larva can be theoretically predicted from the force balance equation at low Reynolds numbers (Happel & Brenner, 1973; Roberts, 1970):

$$s = (\rho - \rho_o)gV/6\pi\eta R,$$

where ρ and V are the average density and volume of the larva, g is the acceleration due to gravity and R is the radius of the equivalent sphere (Stokes' radius) of the larva. s can be evaluated from calculations of R based on the approximation that the shape of the larval body is hydrodynamically equivalent to a prolate spheroid of radius r and major axis l, since R is practically the only variable for a given larva to be determined among others in the above equation when the larval orientation varies with varying θ. For a gastrula with $r = 70\,\mu m$ and the axial ratio $l/r = 1{\cdot}1$, R will increase from $R_V = 1{\cdot}02r$ calculated for the spheroid with the flow of fluid parallel to the major axis to $R_H = 1{\cdot}04$ for that with the flow perpendicular to the axis, as θ varies from 0° to 90°, and will decrease from R_H to R_V as θ varies further from 90° to 180° (Happel & Brenner, 1973). For a pluteus with $r = 80\,\mu m$ and $l/r = 1{\cdot}5$, R will make similar variations between $R_V = 1{\cdot}10r$ and $R_H = 1{\cdot}19r$. Thus, it could be demonstrated that the corrected curves of v *vs* θ, taking account of the effects of variation in s due to changes in the larval orientation as discussed above, would deviate from v in the curves shown in Fig. 6A and Fig. 6C by at most 1 % for gastrulae and 4 % for plutei. The hydrodynamic effects of arms (approx. $50\,\mu m$ in length) of the plutei could also be shown to be negligibly small from calculations similar to those by Emlet (1983).

The swimming vectors of individual larvae in this paper were determined from the swimming paths recorded by time-exposure photography. Since the equations for the theoretical curves are valid, strictly speaking, for calculations based on the instantaneous vectors, the averaged vectors as shown in Fig. 3 may not be suitable for vector calculus. The results shown in Fig. 6A,C, however, were confirmed by a preliminary study of the instantaneous vectors measured by stroboscopic, multi-exposure photography (data are not shown).

The experimental chamber was designed to be somewhat narrow, about three larval diameters, to record two-dimensional swimming paths for ease of analysis.

However, this design of chamber may introduce some physical constraints on swimming speed, because the low Reynolds number, and hence viscous conditions, experienced by microscopic organisms make their performance, such as swimming and falling, sensitive to nearby walls (Winet, 1973). This might explain our observations in other ways than those described above.

Larvae, particularly plutei, moving downwards in the vertical chamber may have been reacting to an encounter with the chamber wall, and therefore may have been closer to the wall than were larvae swimming upwards. Hydrodynamic drag on larvae moving at a particular speed would be larger at a position near the wall than on larvae moving at the same speed but distant from it. This sort of wall effect could be estimated from the equations of drag coefficients for a sphere of radius a, moving parallel to and between two parallel plane walls:

$$D_m = \frac{6\pi\eta a}{1 - 1{\cdot}004(a/L)}$$

when the sphere is moving midway between the walls, i.e. at the same distance L from them and

$$D_n = \frac{6\pi\eta a}{1 - 0{\cdot}563(a/L_1 + a/L_2)}$$

when it is nearer to one of the walls and at distances L_1 and L_2 from them (Happel & Brenner, 1973). Thus the ratio of drags on larvae moving at different distances from the walls can be estimated approximately by D_m/D_n. This value is about 0·5 when $L = 3a$, $L_1 = a$ and $L_2 = 2L - a$. Therefore larvae moving downwards may experience at most twice the drag that those moving upwards experience provided that the positional differences were those speculated above. This might lead, even without physiological regulation, to a reduction of the degree of difference in the swimming speeds between downward and upward swimming, as shown in Fig. 6C.

However, recent experiments (Y. Mogami, S. Hiruma & S. A. Baba, in preparation), in which plutei were put in a similar narrow chamber in ASW containing Percoll to neutralize buoyancy, revealed that independence of swimming velocity from its direction is unlikely to be an artefact of the walls of the experimental chamber. Percoll dissolved in ASW did not significantly change the ionic composition, osmolarity or viscosity of the medium, or alter the geotactic behaviour of larvae, but it selectively reduced the sinking speed to about 1/10 of the control in ASW. The speed of swimming plutei was not significantly different for those moving upwards and downwards; in a typical experiment (one of seven) the average swimming speed was $0{\cdot}29 \pm 0{\cdot}1\,\mathrm{mm\,s^{-1}}$ (mean ± S.D., $N = 22$) upwards ($\theta = 180 \pm 10°$) and $0{\cdot}29 \pm 0{\cdot}1\,\mathrm{mm\,s^{-1}}$ ($N = 15$) downwards ($\theta = 0 \pm 10°$). This result indicates that hydrodynamic effects of the chamber walls under our experimental conditions acted almost uniformly, when averaged among the larvae moving in one direction in the vertical arrangement. Hydrodynamic wall effects should also be considered for larvae moving in the horizontal arrangement. As a result of negative geotaxis, most of the larvae in the horizontal chamber

may reach and swim near the upper wall of the chamber, so that only the speed reduced by the wall effects can be measured in the horizontal arrangement. These may be very different from the values when moving in open water. However, the swimming speed of larvae moving in the horizontal direction in the vertical chamber was not different from that measured in the horizontal chamber (see Fig. 6). Therefore, our speculation, which leads to a suggestion of physiological control for swimming velocity in plutei, seems to be compatible with the hydrodynamic wall effects of both vertical and horizontal chambers.

The small width of the experimental chamber also increases the chance of larvae encountering chamber walls, so that plutei may respond by ciliary reversal followed by a change in swimming direction (Rumrill, Pennington & Chia, 1985), a characteristic feature of the pluteus but not of earlier developmental stages (Baba, 1975; Degawa *et al.* 1986; Baba & Mogami, 1987). The difference in the swimming pattern between gastrula and plutei (Fig. 6) might therefore be explained in part as the result of different responses to encounters with objects, and also of the induced backward swimming of plutei. It should be noted, however, that results almost identical to those shown in Fig. 6C were obtained from analyses of only forward-swimming plutei, distinguished from those swimming backwards by multiple-exposure photography (not shown).

The active control of propulsion suggested here, as well as backward swimming by ciliary reversal, both seen in plutei, may give rise to elaborate swimming patterns adapted for vertical migration, feeding and avoiding predators (Pennington, Rumrill & Chia, 1986; Rumrill *et al.* 1985).

The physiological mechanisms in plutei for the active control of propulsion are far from clear. It is, however, inferred from the direction-dependent regulation of swimming speed described above that propulsive output of the ciliary movement is regulated by some physiological feedback system. One of the possible mechanisms is the control of propulsive output by plutei depending on the swimming direction by reception of the orientation relative to gravity, i.e. gravireception. Another possibility, inferred from the remarkable constancy of the swimming velocity over the whole range of swimming directions, is that plutei control propulsive force through the reception of swimming velocity, i.e. rheoreception, so as to maintain a constant velocity irrespective of swimming direction.

Almost all animals that are capable of gravireception are known to possess statocysts or similar organs (Markl, 1974). However, since no statocyst has been found in echinoid larvae (Hyman, 1955; Markl, 1974), the gravireceptors of plutei, if any, may be different from statocysts. Statocyst-like organelles such as Müller vesicles in *Loxodes* (Fenchel & Finlay, 1986) should therefore be looked for in the pluteus. It should be noted, however, that the theoretical basis for gravireception of the Müller vesicle (Fenchel & Finlay, 1984) may not be directly applicable to plutei because their cells are approx. 5 μm in diameter and therefore rather smaller than the vesicle, which is approx. 7·5 μm and just larger than the theoretical lower limit. Rheoreceptive organs might have to be considered as a possible basis for mechanoreception by the cilia of plutei (Gustafson, Lundgren & Treufelt, 1972;

Strathmann, Jahn & Fonseca, 1972). However, receptors, including gravireceptors and rheoreceptors, which can serve to control swimming velocity, have not been discovered in echinoid larvae. Nevertheless, the pattern of swimming of plutei analysed in the present study can be interpreted as compensation for passive sinking due to gravity by some physiological control mechanisms.

The development of swimming behaviour as described above in sea urchin larvae may be similar for other marine invertebrate larvae, and therefore experiments using sea urchin larvae may provide a model of geotaxis for multicellular micro-organisms whose behaviour depends on the mechanical properties of the larvae, as well as specialized physiological mechanisms including gravireception.

This work was supported by a grant from the Japan Securities Scholarship Foundation.

References

BABA, S. A. (1975). Developmental changes in the pattern of ciliary responses and the swimming behavior in some invertebrate larvae. In *Swimming and Flying in Nature*, vol. 1 (ed. T. Y.-T. Wu, C. J. Brokaw & C. Brennen), pp. 317–323. New York, London: Plenum Press.

BABA, S. A. & MOGAMI, Y. (1987). High time-resolution analysis of transient bending patterns during ciliary responses following electric stimulation in sea-urchin embryos. *Cell Motil. Cytoskel.* **7**, 198–208.

BEAN, B. (1984). Microbial geotaxis. In *Membranes and Sensory Transduction* (ed. G. Colombetti & F. Lenci), pp. 163–198. New York, London: Plenum Press.

CHIA, F.-S., BUCKLAND-NICKS, J. & YOUNG, C. M. (1983). Locomotion of marine invertebrate larvae: a review. *Can. J. Zool.* **62**, 1205–1222.

DEGAWA, M., MOGAMI, Y. & BABA, S. A. (1986). Developmental changes in Ca^{2+} sensitivity of sea-urchin embryo cilia. *Comp. Biochem. Physiol.* **82**A, 83–90.

EMLET, R. B. (1983). Locomotion, drag, and the rigid skeleton of larval echinoderms. *Biol. Bull. mar. biol. Lab., Woods Hole* **164**, 433–445.

FENCHEL, T. & FINLAY, B. J. (1984). Geotaxis in the ciliated protozoan *Loxodes*. *J. exp. Biol.* **110**, 17–33.

FENCHEL, T. & FINLAY, B. J. (1986). The structure and function of Müller vesicles in loxodid ciliates. *J. Protozool.* **33**, 69–76.

FUKUI, K. & ASAI, H. (1980). The most probable mechanism of the negative geotaxis of *Paramecium caudatum*. *Proc. Japan Acad. Ser.* B **56**, 172–177.

FUKUI, K. & ASAI, H. (1985). Negative geotactic behaviour of *Paramecium caudatum* is completely described by the mechanism of buoyancy-oriented upward swimming. *Biophys. J.* **47**, 479–482.

GUSTAFSON, T., LUNDGREN, B. & TREUFELT, R. (1972). Serotonin and contractile activity in the echinopluteus. A study of the cellular basis of larval behaviour. *Expl Cell Res.* **72**, 115–139.

HAPPEL, J. & BRENNER, H. (1973). *Low Reynolds Number Hydrodynamics*. Leyden: Noordhoff Int. Publish.

HYMAN, L. H. (1955). *The Invertebrates*, vol. VI, *Echinodermata*. New York: McGraw-Hill.

MARKL, H. (1974). The perception of gravity and of angular acceleration in invertebrates. In *Handbooks of Sensory Physiology*, vol. VI/1 (ed. H. H. Kornhuber), pp. 17–74. Berlin: Springer-Verlag.

PENNINGTON, J. T. & EMLET, R. B. (1986). Ontogenic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus* (Eschscholtz): occurrence, causes, and probable consequences. *J. exp. mar. Biol. Ecol.* **104**, 69–95.

PENNINGTON, J. T., RUMRILL, S. S. & CHIA, F.-S. (1986). Stage-specific predation upon embryos and larvae of the pacific sand dollars, *Dendraster excentricus*, by 11 species of common zooplanktonic predators. *Bull. mar. Sci.* **39**, 234–240.

ROBERTS, A. M. (1970). Geotaxis in motile micro-organisms. *J. exp. Biol.* **53**, 687–699.

RUMRILL, S. S., PENNINGTON, J. T. & CHIA, F.-S. (1985). Differential susceptibility of marine invertebrate larvae: laboratory predation of sand dollar, *Dendraster excentricus* (Eschscholtz), embryos and larvae by zoeae of the red crab, *Cancer productus* Randall. *J. exp. mar. Biol. Ecol.* **90**, 193–208.

STRATHMANN, R. R., JAHN, T. L. & FONSECA, J. R. C. (1972). Suspension feeding by marine invertebrate larvae: Clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull. mar. biol. Lab., Woods Hole* **72**, 505–519.

WINET, H. (1973). Wall drag on free-moving micro-organisms. *J. exp. Biol.* **59**, 753–766.

YASUMASU, I. (1963). Geotaxis of swimming sea-urchin larvae. *Zool. Mag.* **72**, 160–162.