J. exp. Biol. ***176****, 207–221 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

SEPARATING THE EFFECTS OF TEMPERATURE AND VISCOSITY ON SWIMMING AND WATER MOVEMENT BY SAND DOLLAR LARVAE (*DENDRASTER EXCENTRICUS*)

R. D. PODOLSKY*

Department of Zoology NJ-15, University of Washington, Seattle, WA 98195, USA

and R. B. EMLET*,†

Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA

Accepted 20 October 1992

Summary

The small size and slow movement of aquatic, microscopic organisms means that the viscosity of water has a predominant influence on their motion. Temperature, through its effects on physiological processes, also influences motion. Because water viscosity is physically coupled to temperature, changes in temperature can influence the activity of microscopic organisms through both physiological and physical means. To partition these effects, we artificially altered seawater viscosity and, at two temperatures, we measured swimming speed and water movement by larvae of the sand dollar *Dendraster excentricus*. Over an environmentally relevant, 10-degree drop in water temperature (22 to 12°C), swimming speed was reduced by approximately 40% and water movement was reduced by 35%. 40% of the decrease in swimming speed and 55% of the decrease in water movement were accounted for by increases in viscosity alone. The physical effects of viscosity can therefore make up a large component of the effect of temperature on activity of microscopic organisms. If uncorrected for effects of viscosity, temperature coefficients such as Q_{10} values can overestimate the influence of temperature on the physiological processes that underlie the generation of motion at small spatial scales. These changes in viscosity may cause substantial reductions or increases in swimming and feeding rates that are biologically relevant. Environmental variation in viscosity due to temperature fluctuations could lead to temperature responses or adaptations that are nonphysiological.

Introduction

Most microscopic organisms live in aqueous media. For these organisms, motility depends both on internal, physiological processes and on the physical properties of the

*The authors consider their efforts to be equal in this work.

†Present address: Institute of Marine Biology and Department of Biology, University of Oregon, Charleston, OR 97420, USA.

Key words: viscosity, larvae, swimming, echinoid, cilia, temperature, Q_{10}, *Dendraster excentricus*.

fluid environment. Forces generated by reciprocating structures (flagella, cilia, setae or the whole body) produce movement which is fueled by biochemical processes, by energy stored in phosphate bonds. However, the translation of force into motion depends on how these structures interact with the fluid. Because of the small size and slow movement of microscopic organisms, the physical properties of water (e.g. density and dynamic viscosity) have a predominant influence on an organism's motion and are considered to be a major force in the evolution of their various modes of locomotion and feeding (LaBarbera, 1984; Strickler, 1984; Emlet and Strathmann, 1985; Power, 1989; Denny, 1990).

Both the physiological and physical components of aquatic locomotion are strongly influenced by temperature. Temperature affects physiological function mainly through its effect on rates of biochemical reaction (Hochachka and Somero, 1984). Although temperature only weakly affects the density of water, it has a strong effect on water's dynamic viscosity (hereafter, viscosity, μ), which more than doubles between tropical and arctic ocean temperatures (at a salinity of 30‰, μ=0.863cP at 30˚C and μ=1.875cP at 0˚C; Dorsey, 1968). Although the inverse temperature–viscosity relationship is a universal feature of aquatic systems, little research has been done to partition the biological consequences of simultaneous changes in temperature and viscosity.

Because individuals often experience a broad range of environmental temperatures, and because temperature is the environmental variable strongly associated with geographical variation within and among species, temperature effects have been studied more extensively than other abiotic influences (Wieser, 1973; Cossins and Bowler, 1987). Given that both the physiological and physical components of temperature change can modify performance in aquatic environments, each may serve as a selective agent. Organisms have evolved adaptations to temperature that help maintain physiological function through various adjustments in enzyme systems (Hochachka and Somero, 1984). In contrast, adaptations to environmental changes in viscosity associated with temperature have not been explored.

The effects of temperature on activity are traditionally summarized by the Q_{10}, a coefficient that gives the relative change in a rate over a specified 10˚C change in temperature (Schmidt-Nielsen, 1990). Often Q_{10} is used to infer the temperature-dependence of physiological processes underlying activity (Cossins and Bowler, 1987). For small-scale processes (e.g. water movement by cilia) where viscosity of the fluid affects rates of movement, measurements of the effect of temperature on activity include both physiological and physical components. Temperature coefficients that do not consider the effects of viscosity may therefore overestimate the physiological effects of temperature.

To partition the effects of temperature and viscosity, we used a simple technique for artificially altering seawater viscosity. We measured swimming activity and water movement in larvae of the sand dollar *Dendraster excentricus* (Eschscholtz). Small ciliated larvae of *Dendraster* provide a useful model organism for studying the interactive effects of temperature and viscosity because: (1) mechanisms of ciliary propulsion involve viscous forces (Sleigh and Blake, 1977), (2) larvae can be tethered in place for measurements of water movement (Emlet, 1990), (3) metabolic responses to temperature

change have been examined (McEdward, 1984, 1985), (4) effects of temperature on water movement are likely to have consequences for feeding and life-history characteristics related to feeding development (Strathmann, 1971, 1985), and (5) these effects can be quantified and easily related to body form (Hart, 1991). By adjusting the viscosity of sea water at high temperature to match that at low temperature, we examined the effects of temperature, with and without changes in viscosity, on larval swimming speed and water movement. This comparison allowed us to estimate the relative contributions of physiology and viscosity to changes in activity within a range of temperatures and viscosities to which larvae are normally exposed.

Materials and methods

Study organism

In July 1991, we collected adult sand dollars (*Dendraster excentricus*) from an intertidal area at Olga on Orcas Island in San Juan County, Washington, USA, and stored them in flowing seawater tanks at the Friday Harbor Laboratories, San Juan Island. To induce spawning of gametes, adults were injected with 2ml of 0.55mol l^{-1} KCl solution. Before fertilization, eggs were rinsed twice with sea water that had been filtered with a bag filter with mesh less than 10 μm. Larvae were cultured at room temperature (approximately 20˚C) and fed every 2–3 days from cultures of the flagellates *Dunaliella tertiolecta* and *Rhodomonas lens*. Experiments were performed with larvae that were 12–19 days old at the six- to eight-arm stage of development (Strathmann, 1987). The water currents used by larvae in swimming and feeding are generated by a ciliated band that runs in a convoluted path around the larval arms. Nomenclature for larval arms is given in Fig. 1 and is according to Mortensen (1921).

Manipulation of seawater viscosity

In the experiments described below, we compared larval activity under three treatments: 0.22 μm-filtered sea water at 22˚C (T22), at 12˚C (T12) and at 22˚C with viscosity adjusted to that at 12˚C (T22/μ12). Larval development is normal within this temperature range for populations of *D. excentricus* in Puget Sound (H. Fujisawa, unpublished data; McEdward, 1985).

We adjusted seawater viscosity by adding polyvinyl pyrrolidone (PVP; M_r 360000; Sigma Chemical Co.). PVP has been used to increase longevity of movement in preparations of demembranated flagellar organelles (Goldstein, 1974) and is commonly used to increase fluid viscosity for ciliary and flagellar studies (e.g. Baba and Hiramoto, 1970; Belas *et al.* 1986). PVP is a suitable agent for manipulating viscosity because PVP solutions (1) show constant viscosity over a wide range of shearing stresses (Baba and Hiramoto, 1970), and (2) do not affect fertilization rates of sand dollar gametes (R. Podolsky and C. Lee, unpublished data), a standard assay of chemical toxicity (Dinnell *et al.* 1987). Embryos and larvae of *Dendraster* raised in PVP solutions developed normally with no apparent increases in mortality. A concentration of 1.44 g l^{-1} PVP was needed to adjust the viscosity of 22˚C sea water (μ=1.02cP for 30‰ sea water; Dorsey,

1968) to that of 12°C sea water (μ=1.30cP), as determined using a falling ball viscometer (Gilmont Instruments, GV-2100). To remove low molecular weight impurities and to make the solutions isosmotic with sea water, untreated filtered sea water and stock PVP solutions ($4\,g\,l^{-1}$) were held in dialysis tubing in flowing sea water for 24h prior to experiments. For experiments, dialyzed stock PVP solution was diluted with filtered sea water to the appropriate concentration.

Measurements of swimming speed

To measure larval swimming speed we took advantage of the tendency for sand dollar larvae to swim upwards in the water column (Pennington and Emlet, 1986; Mogami *et al.* 1988). A swimming chamber was constructed from a polystyrene culture flask (70mm×35mm×12mm) with horizontal lines etched every 3mm along the front face. The flask was held upright and submerged to the neck in a constant-temperature bath. Through a conduit of polyethylene tubing that entered the bottom of the chamber, we introduced a single larva to the chamber, allowed it to ascend for approximately 30mm, and then measured its speed over a vertical distance of 9mm near the middle of the chamber. This area was magnified on a video screen by using a camera with a macro lens fixed about 10cm from the chamber. A fiber-optic light positioned above the chamber was used to illuminate the larva. The time to cross the 9mm distance was measured with a hand-held stopwatch. To reduce variation caused by effects of wall-induced drag (Winet, 1973), we measured larvae that were in the plane of focus at the center of the chamber. Larvae used in experiments were acclimated to the appropriate treatment temperature for 2–4h before measurements. Individuals that showed unusual or nondirectional movement in the chamber were rejected (fewer than 5% of the total).

We measured swimming speeds of 40 larvae in each of the three treatments described above. This procedure was replicated over 3 days (at larval ages 12, 13 and 15 days), with the order of treatments varied among days to conform to a Latin square (total N=360 larvae). The chamber was flushed and filled with new solution after every 20 larvae; we treated the average speed of each group of 20 larvae as a replicate. We performed a two-way analysis of variance (ANOVA) on swimming speed with day and treatment as the two factors. To test for an effect of time of day, at the end of each day's treatments we repeated measurements for the first treatment of that day on 20 additional larvae. Using a *t*-test before proceeding with the ANOVA, we compared each of these 'control' groups with the respective first treatment groups. Because we predicted the order of treatment effects *a priori* (T22>T22/μ12>T12), we used the Tukey test with an adjusted alpha=0.025 (Zar, 1984) and compared the means of adjacent treatments.

Measurements of water movement

High-speed video recordings ($200 frames\,s^{-1}$, NAC camera and recorder) of water movement created by tethered echinoplutei were made with a photomicroscope. The same treatment conditions were used as those in swimming studies (i.e. T22, T22/μ12, T12). By repeating the treatment of filtered sea water at 22°C (T22 no. 2 after the PVP treatment, we tested whether the effects of exposure to PVP were immediately reversible.

Larvae were filmed in a chamber (volume approximately 1ml) consisting of a polyethylene ring 19mm in diameter and 3mm high sealed to the upper surface of a cooling slide. The cooling slide consisted of two coverslips sealed to a hollowed-out and plumbed brass plate (a modified version of the cooling slide described by Stephens, 1973). The cooling slide was connected to a refrigerated, circulating water bath which controlled the temperature of the water in the filming chamber by conduction through the chamber's coverslip bottom. Prior to filming and between treatments the temperature of the water bath and filming chamber were monitored to the nearest 0.1˚C with thermistor probes (Yellow Springs Instruments Co.).

Each larva was held in place by drawing one of its posterodorsal arms into a suction pipette with a diameter of approximately 30 μm (Fig. 1). The larval arm fitted snugly into the pipette and suction retained the larva in place during filming. The larva was positioned with the dorsal surface up, in the center of the chamber, approximately 0.5mm from the chamber bottom. Polystyrene beads (2 μm diameter, Duke Scientific, Inc.) were used as fluid markers. After filming the larva in one solution, the chamber was gently flushed four or five times with 1ml volumes of the next treatment solution. The chamber was then refilled with test solution and allowed to equilibrate to the test temperature. The tethered larva was often moved during the flushing of the chamber and usually had to be reoriented prior to filming. However, no larvae included in the analysis were lost from the suction pipette during changes of chamber fluid. The order of treatments for all larvae was T22 no. 1 followed by T22/μ12. Four of the larvae then had T22 no. 2 followed by T12, whereas two larvae had T12 followed by T22 no. 2.

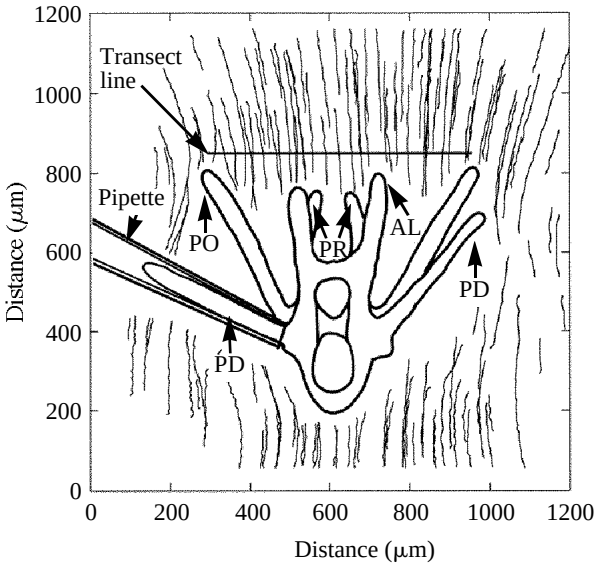

Fig. 1. Digitized video image of a tethered larva of *Dendraster excentricus.* Lines are paths of 2 μm beads, moving from top to bottom of the figure. Particle velocities were determined from particles crossing the transect line, 50 μm upstream of the postoral (PO), anterolateral (AL) or preoral (PR) arms. The larva is held in a horizontal viewing plane by a suction pipette over the left posterodorsal arm (PD).

Data analysis

High-speed video images of water movement by six tethered larvae were analyzed with Expert Vision software (Motion Analysis Inc.). One to two minutes of recorded images were processed to determine particle paths (*x,y* coordinates at each 0.01s, Fig. 1). The data on particle paths were processed by our own computer program, which computed particle velocities from the positions of particles before and after they crossed a transect line and from the time elapsed. The transect line was oriented orthogonally to the direction of water current and was 50 μm upstream of the anteriormost arm tip. For a given larva, the same arm tip (either a postoral or anterolateral arm tip) was used across treatments. The maximum length of the transect line was the distance between the postoral arm tips (Fig. 1). Because a larva often varied in position in the video fields of different treatments, particle paths were analyzed only for the part of the transect line that was common to all four treatments.

For each treatment we constructed a plot of the velocity of particles as a function of position along the transect line. Plots of all treatments for a given larva were then superimposed by adjusting for position of the larva. Regression equations were fitted to the velocity data (e.g. Fig. 3). We used a first-, second- or third-order regression model, depending on the distribution of particle velocities, but for any given larva the same order regression consistently fitted the velocity distributions best. Data for each treatment are presented in two forms. (1) The area enclosed by the regression line and the transect line is an estimate of flux in the plane of the particle paths over the length of the transect line ('area-flux', $\mu m^2 s^{-1}$) (equivalent to the integral of the fitted regression equation). (2) This area divided by the length of the transect line gives an average velocity along the measured line. Because the underlying distribution of averages was expected to approximate normality, we used a two-way ANOVA without replication to analyze statistically the average velocities. As in the swimming speed studies, we predicted the order of treatment effects *a priori* and carried out paired *t*-tests between treatments T22 *versus* T22/μ12 and treatments T22/μ12 *versus* T12.

Results

Swimming speed

Larvae swam more slowly both at lower temperature and at higher viscosity (Fig. 2). On average, swimming speed decreased by 39% when the temperature of untreated sea water was reduced from 22°C ($439\pm16\,\mu m\,s^{-1}$) to 12°C ($266\pm6\,\mu m\,s^{-1}$, mean ± S.E. across all replicates). This change in speed presumably reflects the effects of temperature on both physiology and viscosity. When we adjusted only the viscosity of 22°C sea water without a change in temperature (T22/μ12), mean swimming speed reduced to $369\pm11\,\mu m\,s^{-1}$, a decline of 16%. Thus, about 40% of the decline in speed was attributable to changes in seawater viscosity and 60% to other effects of temperature. The ANOVA showed a significant effect of treatment ($F_{2,9}$=56.9, P<0.0001), with no significant effects of day or treatment times day. The order of treatment means conformed to our prediction (T22>T22/μ12>T12); in both cases adjacent means were significantly

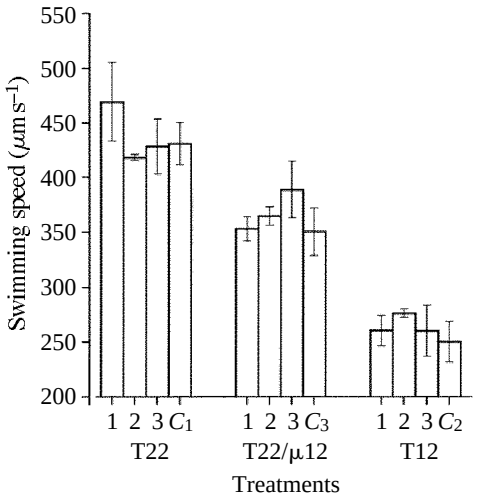

Fig. 2. Swimming speed as a function of treatment for populations of larvae of *Dendraster excentricus* measured on each of 3 days. For the three treatments the numbered bars show the mean swimming speed and S.E. for two replicate (20 larvae per replicate) populations. Numbers below the bars indicate the day of the experiment. The bars labeled with *C* and a number represent 'controls' for time of day and report the mean and S.E. for 20 additional larvae. The number associated with *C* indicates the day to which the 'control' corresponds (see Materials and methods). Treatments: T22, sea water at 22˚C; T22/μ12, sea water at 22˚C with viscosity adjusted to that at 12˚C; T12, sea water at 12˚C.

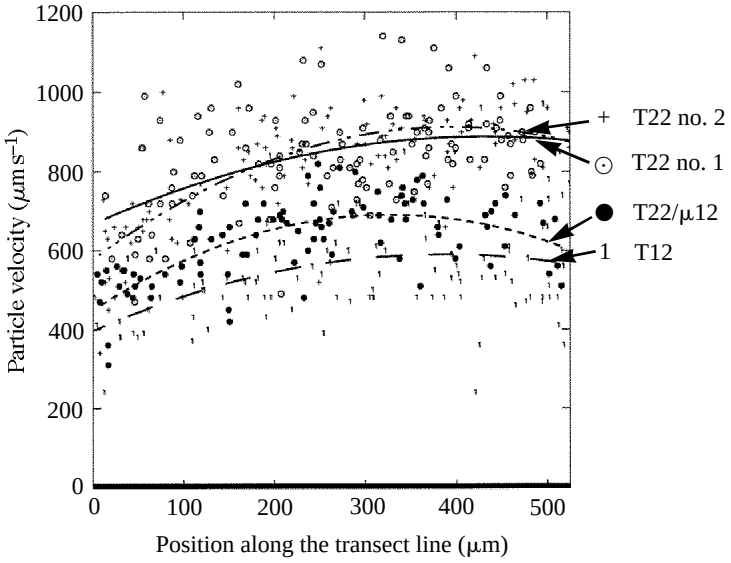

Fig. 3. Particle velocities for particles crossing the transect line for one larva of *Dendraster excentricus* (see Fig. 1). For this particular larva (no. 3 in Table 1), second-order regressions were fitted to the velocity data. Symbols for the different treatments are identified in the figure. Treatments: T22 no. 1 and T22 no. 2, replicate treatments of sea water at 22˚C; T12, sea water at 12˚C; T22/μ12, sea water at 22˚C with viscosity adjusted to that at 12˚C.

Table 1. *Water flux and water velocity for different larvae and treatments of tethered echinoplutei of* Dendraster excentricus

Larva number	Transect line (μm)	Measure	Treatments				Regression order
			T22 no. 1	T22 no. 2	T22/μ12	T12	
1	516	Flux	0.436	0.386	0.266	0.224	1
		Velocity	845	748	514	434	
2	525	Flux	0.382	0.374	0.328	0.285	1
		Velocity	726	712	624	542	
3	500	Flux	0.414	0.415	0.317	0.272	2
		Velocity	828	829	633	544	
4	326	Flux	0.265	0.266	0.244	0.200	2
		Velocity	811	814	748	613	
5	310	Flux	0.233	0.201	0.177	0.133	2
		Velocity	751	647	570	429	
6	338	Flux	−	0.206	0.200	0.142	3
		Velocity	−	608	592	419	
Mean area-flux			0.346	0.308	0.255	0.209	
S.E.			0.041	0.039	0.025	0.026	
Mean water velocity				759.3*	613.5	496.8	
S.E.				32.9†	32.0	32.8	

*Value was calculated from (average velocity for five values of T22 no. 1 plus the average velocity for the six values for T22 no. 2) divided by 2.

†Value was calculated from two means for T22 no. 1 and T22 no. 2.

Different larvae are shown with their own transect line lengths (μm), estimates of the area-flux (flux in $mm^2\,s^{-1}$) and average water velocity (velocity in $\mu m\,s^{-1}$).

Also reported is the order of the regression equations fitted to treatments for each larva. Treatments: T22 no. 1 and T22 no. 2, sea water only at 22°C; T12, sea water only at 12°C; T22/μ12, sea water at 22°C with viscosity adjusted to that of sea water at 12 °C.

different (T22 *versus* T22/μ12: $q_{9,2}$ t=6.09, P<0.005; T22/μ12 *versus* T12: $q_{9,2}$=8.94, P<0.001).

The ‘standard’ Q_{10} value, which incorporates all effects of temperature on swimming speed, was 1.65 for the temperature interval 12–22˚C. The ‘viscosity-free’ Q_{10} value, which is the ratio of swimming speeds in treatments T22/μ12 and T12, was 1.39. This comparison holds seawater viscosity constant and includes only other effects of temperature. Using the ‘standard’ Q_{10} value to infer physiological effects of temperature on larval movement would overestimate the effect of temperature on physiology by 67%.

Water movement

Changes in temperature and viscosity probably affect swimming speed through changes in the amount of water moved by cilia per unit time. Our measurements of water movement, both area-flux and average water velocity, decreased substantially with decreasing temperature and increasing viscosity (Fig. 3, Table 1). The mean velocity in the region just upstream of the larval arms decreased by 35% over the 10-degree

temperature change, from 759 $\mu m s^{-1}$ (±33 $\mu m s^{-1}$, S.E.) at 22°C to 497 $\mu m s^{-1}$ (±33 $\mu m s^{-1}$, S.E.) at 12°C (Table 1). For larvae at 22°C in sea water adjusted to the viscosity of sea water at 12°C, mean water velocity was 614 $\mu m s^{-1}$ (±32 $\mu m s^{-1}$, S.E.), a drop of 19% relative to sea water at 22°C (Table 1). Changes in viscosity thus account for 55% of the overall drop in water velocity over the 22–12°C temperature range. Treatments differed significantly in average water velocity (ANOVA, $F_{3,5}$=31.7, P<0.001). A paired t-test between treatments T22 no. 1 and T22 no. 2 showed no significant difference for the five larvae where both values were available (t=1.75, d.f.=4, P=0.16), indicating no short-term effects of exposure to PVP. Data from the two T22 treatments were pooled for further comparisons. One-tailed, paired t-tests confirmed our predictions about the order of treatment means (T22 *versus* T22/μ12: t=3.33, d.f.=5, P<0.01; T22/μ12 *versus* T12: t=7.52, d.f.=5, P<0.0005).

A 'standard' Q_{10} of 1.53 was calculated for average water velocity from data for seawater treatments alone (12–22°C). However, if viscosity is held constant, a Q_{10} of 1.24 is obtained. For water movement, use of the standard Q_{10} value would overestimate the effect of temperature on physiology by 120%.

Discussion

Partitioning the effects of temperature and viscosity

For ectothermic animals, environmental temperature can affect performance in activities such as locomotion, feeding and reproduction. Studies commonly attribute such effects to the influence of temperature on biochemical processes, particularly the catalytic and regulatory properties of enzymes (Wieser, 1973; Hochachka and Somero, 1984). Our results show that, for microscopic organisms, the physical effects of viscosity can also constitute a large component of the effect of temperature on activity. For larvae of *Dendraster*, change in viscosity alone accounted for 40% of the change in swimming speed and more than 50% of the change in water movement when temperature was reduced by 10°C. The disagreement between these two measures may have resulted from comparing freely swimming with tethered larvae. Holding a larva in place increases the shear gradient created at the level of individual cilia (Emlet, 1990) and this may result in the greater apparent contribution of viscosity to changes in water movement. Nearby walls in the small tethering chamber may also have increased the effects of viscosity relative to those in the larger swimming chamber. In the only comparable study we could find, Mitchell *et al.* (1991) reported that viscosity accounted for about 26% of the change in swimming speed over a 30°C change in temperature for the purple sulfur bacterium *Chromatium minus*. Although this number is somewhat lower than our values, their study included a range of higher temperatures (15–45°C) over which changes in viscosity are relatively small (Dorsey, 1968).

Our results underscore a problem of using temperature coefficients, such as Q_{10} values, to infer effects of temperature on whole-animal physiology (Cossins and Bowler, 1987). 'Standard' Q_{10} values in our study severely overestimated the influence of temperature on the physiological processes that underlie the generation of force by cilia. In addition, the

relative importance of viscosity *versus* physiology may depend on the range of temperatures considered, as noted above for the study by Mitchell *et al.* (1991). For example, it is commonly observed that the magnitude of Q_{10} for movement of microscopic organisms decreases at higher temperatures (Gray, 1923; Lee, 1954; Sleigh, 1956). Although this general result is predicted from thermodynamic considerations (Schmidt-Nielsen, 1990, p. 575), viscosity also declines exponentially with increasing temperature (Dorsey, 1968), resulting in smaller viscosity changes per degree at higher temperatures. Furthermore, in a study preliminary to ours, R. D. Podolsky, D. J Mense and A. B. Kettle (unpublished data) found that a unit change in viscosity had a greater effect on swimming speed at higher viscosities than at lower viscosities. Thus, the relative importance of viscosity in modifying activity is likely to increase at lower temperatures.

Environmental changes in viscosity

Why the effects of environmental viscosity have been ignored is unclear, given the broad base of knowledge that suggests their importance. First, viscosity is widely recognized as dominating hydrodynamic processes at small scales. Mechanisms of locomotion and feeding in microorganisms depend primarily on the viscous properties of water (e.g. Brennen and Winet, 1977; Purcell, 1977; Wu, 1977; Koehl and Strickler, 1981), as does filter-feeding in large and small animals from diverse phyla (Jørgensen, 1983; LaBarbera, 1984; Shimeta and Jumars, 1991). Second, temperature has been known to influence the motility of microscopic organisms (Lee, 1954; Clayton, 1958; Castenholz, 1973; Maeda *et al.* 1976; Hidu and Haskin, 1978). Third, manipulations of fluid viscosity have been used to elucidate the properties of cilia and flagella under large viscous loads (Yoneda, 1962; Brokaw, 1966; Baba and Hiramoto, 1970; Berg and Turner, 1979; Gheber and Priel, 1990). In such studies, adjustments to viscosity are normally well beyond the range found in natural environments. These disciplines, despite their extensive development, have not explored the biological implications of environmental viscosity change associated with changes in temperature.

Recently, Jørgensen *et al.* (1986, 1990) have examined the effect of temperature on flow rates and ciliary beat frequencies in the filter-pump mechanism of the mussel *Mytilus edulis*. They noted a close inverse linear relationship between pumping rate and kinematic viscosity (=dynamic viscosity/density) and argued that there was little residual variation to be explained by other factors, such as the temperature-dependence of ciliary beating. Because the dynamic viscosity of sea water changes much more than its density as temperature shifts, their results imply that dynamic viscosity was the major factor that influenced pumping rate. However, kinematic viscosity was manipulated only through temperature change, and thus the effects of kinematic viscosity may be confounded with other effects of temperature.

The temperature difference considered in this study (12 *versus* 22˚C) is within the natural range that can be experienced by an individual *Dendraster* larva during the 1–2 months that it spends feeding near the ocean surface (Emlet, 1986). Though we have begun to explore the effect of environmental viscosities on swimming, we know little about the ecological consequences of this effect. For a planktonic organism, swimming speed may be important for regulating vertical position in the water column

(Mileikovsky, 1973; Chia *et al.* 1984). Even more important than viscosity's effect on swimming is its potential effect on feeding. In many planktonic filter-feeders, the swimming currents generated by beating cilia or appendages are also used for collecting food particles. *Dendraster* larvae concentrate food mainly by reversing cilia locally to beat towards the mouth when particles are detected (Strathmann, 1971; Hart, 1991). Thus, any effects of viscosity exhibited in swimming may also affect feeding rate or efficiency. Viscosity could affect the efficiency of suspension feeding by altering the rates of water processing (see also Jørgensen *et al.* 1990) or the distance at which particles can be detected. High viscosity increases the distance at which objects influence each other's flow fields and may potentially aid mechanoreception (Zaret, 1980). Because larval growth and development can depend on natural food availability (Paulay *et al.* 1985; Olson and Olson, 1989), these could be slowed by an effective reduction in particle clearance rates at higher viscosity.

Potential adaptations to changes in viscosity

Given time to acclimatize to temperature changes within a normal range, many marine organisms show nearly complete physiological compensation that allows maintenance of function (Hochachka and Somero, 1984; Clarke, 1991). However, viscosity changes associated with temperature involve consequences that may not be solvable by physiological adaptation and may require other adaptations, such changes in behavior or structure. In larvae of the mosquito *Culicoides variipennis*, the amplitude of body undulations depended on fluid viscosity (Linley, 1986). This response appeared to be a behavioral adjustment in swimming mode because changes occurred gradually, not simultaneously, with movement through a low-to-high viscosity interface. Similarly, increased viscosity alters the metachronal wavelength of ciliary beating in *Paramecium* (Machemer, 1972, 1974); whether this response is active or simply a physical consequence of the ciliary field interacting with a more viscous medium is not known. Because higher viscosity may require a greater power output (e.g. cilium power is proportional to water viscosity, and angular velocity^2; Sleigh and Blake, 1977), such behavioral responses may be more important in conserving energy than in maintaining a given swimming speed.

Plankton experience less drag and sink more quickly in lower- than in higher-viscosity water (Smayda, 1970). This has been used to explain the common observation that plankton from tropical waters have body forms that increase drag (Hardy, 1965). Hebert (1978) suggested that seasonal changes in *Daphnia* morphology involved changes in the size of propulsive muscles in response to seasonal shifts in water viscosity. Nevertheless, the role of viscosity in determining planktonic body form has not been convincingly established (Smayda, 1970; Vogel, 1981). For larger organisms that create ciliary currents through channels or ducts, the results of Jørgensen *et al.* (1990) suggest that changes in ciliary beat might be insufficient to overcome increases in viscous resistance that are experienced at lower temperatures. In this case, adjustments of channel dimensions may be more effective than changes in propulsive mechanisms (e.g. see Reiswig, 1975).

Anatomical adaptations to viscosity could include changes in the distribution of

propulsive structures (Emlet, 1991) or in the contractile elements within these structures (Sleigh, 1989). A particularly interesting example is the bacterium *Vibrio parahaemolyticus*, which can be induced to grow lateral flagella when the viscosity of the culture medium is increased from 1–40cP (Belas *et al.* 1986; McCarter *et al.* 1988). This very large change in viscosity presumably reflects real or apparent changes in the viscosity of the aqueous environment as the organism shifts from free living to surface dwelling (Belas *et al.* 1986). Polar flagella are sufficient for locomotion in the free-living state under normal viscosities, while lateral flagella permit enhanced rates of locomotion under high-viscosity conditions (Atsumi *et al.* 1992).

McEdward (1985) examined developmental rates and metabolic rates of larvae of *Dendraster excentricus* at 12, 17 and 22˚C. He found that rates of development and growth were strongly temperature-dependent, but that larval form was similar at equivalent developmental stages regardless of temperature for larvae raised on an abundant food source. Over the temperature range 12–22˚C, McEdward reported a 93% increase in electron transport activity, a measure of potential metabolic rate, and predicted that larvae would show morphological changes to increase feeding ability at high temperatures when food was more limited. Our results suggest that reduction in water viscosity with increase in temperature could effectively increase clearance rates. If, as a first approximation, we assume that the rate of water movement accurately reflects the rate at which food is collected (Strathmann, 1971), then feeding rate would increase 53% over the temperature interval 12–22˚C, with about 55% of this change being due to the decrease in viscosity. This argument suggests that the increase in metabolic demand could be partially offset by an increased rate of water processing and food capture. Nevertheless, given McEdward's measurements, a gap would still exist between energy supply (food) and energy demand and other means of compensation may be expected. Larvae of *Dendraster* and other echinoids respond to low food concentrations by developing relatively longer arms, thus increasing the length of the ciliated band used in food collection (Boidron-Metairon, 1988; Hart and Scheibling, 1988; Strathmann *et al.* 1992). Aside from changes in morphology that may increase feeding rate, a down-regulation of metabolic rate might be expected under conditions of food limitation and high temperature.

Further investigation of the effects of viscosity on movement of water and locomotion of microscopic organisms is needed to show how performance is affected by changes in this important environmental variable. Future studies examining the effect of temperature on small-scale processes should also consider the concomitant changes in viscosity. While these changes in viscosity are not great enough to alter the relative importance of inertial and viscous forces (i.e. Reynolds number), they may cause substantial changes in swimming and feeding rates that are biologically relevant.

We thank the Director of Friday Harbor Laboratories, University of Washington, for making space and equipment available to conduct this research. This research was supported by setup funds from the University of Southern California and by NSF grant BSR-9058139 to R.B.E. and an NSF pre-doctoral graduate fellowship to R.D.P. We are grateful to R. Strathmann for suggestions and comments during the course of this work,

and to D. Mense and A. Kettle for earlier contributions of ideas. N. Applemans, J. Morin, R. Strathmann and the UCLA seminar in Invertebrate Biology offered helpful comments on an earlier version of this manuscript.

References

ATSUMI, T., MCCARTER, L. AND IMAE, Y. (1992). Polar and lateral flagellar motors of marine *Vibrio* are driven by different ion-motive forces. *Nature* **355**, 182–184.

BABA, S. A. AND HIRAMOTO, Y. (1970). A quantitative analysis of ciliary movement by means of high-speed microcinematography. *J. exp. Biol.* **52**, 675–690.

BELAS, R., SIMON, M. AND SILVERMAN, M. (1986). Regulation of lateral flagella gene transcription in *Vibrioparahaemolyticus*. *J. Bacteriol.* **167**, 210–218.

BERG, H. C. AND TURNER, L. (1979). Movement of microorganisms in viscous environments. *Nature* **278**, 349–351.

BOIDRON-METAIRON, I. F. (1988). Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschshcholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. *J. exp. mar. Biol. Ecol.* **119**, 31–41.

BRENNEN, C. AND WINET, H. (1977). Fluid mechanics of propulsion by cilia and flagella. *A. Rev. Fluid Mech.* **9**, 339–398.

BROKAW, C. J. (1966). Effects of increased viscosity on the movements of some invertebrate spermatozoa. *J. exp. Biol.* **45**, 113–139.

CASTENHOLZ, R. W. (1973). Movements. In *The Biology of Blue-Green Algae* (ed. N. G. Carr and B. A. Whitton), pp. 320–339. Berkeley: University of California Press.

CHIA, F.-S., BUCKLAND-NICKS, J. AND YOUNG, C. M. (1984). Locomotion of marine invertebrate larvae: a review. *Can. J. Zool.* **62**, 1205–1222.

CLARKE, A.(1991). What is cold adaptation and how should we measure it? *Am. Zool.* **31**, 81–92.

CLAYTON, R. K. (1958). On the interplay of environmental factors affecting taxis and motility in *Rhodospirilium rubrum*. *Arch. Mikrobiol.* **29**, 189–212.

COSSINS, A. R. AND BOWLER, K. (1987). *Temperature Biology of Animals.* New York: Chapman and Hall.

DENNY, M. W. (1990). Terrestrial *versus* aquatic biology: The medium and its message. *Am. Zool.* **30**, 111–121.

DINNELL, P. A., LINK, J. M. AND STOBER, Q. J.(1987). Improved methodology for a sea urchin sperm cell bioassay for marine waters. *Arch. Environ. Toxicol.* **16**, 23–32.

DORSEY, N. E. (ed.) (1968). *Properties of Ordinary Water-Substance*. New York: Hafner Publications.

EMLET, R. B. (1986). Larval production, dispersal and growth in a fjord: a case study on larvae of the sand dollar *Dendraster excentricus*. *Mar. Ecol. Prog. Ser.* **31**, 245–254.

EMLET, R. B. (1990). Flow fields around ciliated larvae: effects of natural and artificial tethers. *Mar. Ecol. Prog. Ser.* **63**, 211–225.

EMLET, R. B. (1991). Functional constraints on the evolution of larval forms of marine invertebrates: experimental and comparative evidence. *Am. Zool.* **31**, 707–725.

EMLET, R. B. AND STRATHMANN, R. R. (1985). Gravity, drag and feeding currents of small zooplankton. *Science* **228**, 1016–1017.

GHEBER, L. AND PRIEL, Z. (1990). Ciliary activity under normal conditions and under viscous load. *Biorheology* **27**, 547–557.

GOLDSTEIN, S. F. (1974). Isolated, reactivated and laser-irradiated cilia and flagella. In *Cilia and Flagella* (ed. M. A. Sleigh), pp. 111–130. London: Academic Press.

GRAY, J.(1923). The mechanism of ciliary movement. III. The effect of temperature. *Proc. R. Soc. Lond. B* **95**, 6–15.

HARDY, A. (1965). *The Open Sea: Its Natural History*. Cambridge: The Riverside Press.

HART, M. W.(1991). Particle captures and the method of suspension feeding by echinoderm larvae. *Biol. Bull. mar. biol. Lab., Woods Hole* **180**, 12–27.

HART, M. W. AND SCHEIBLING, R. E. (1988). Comparing shapes of echinoplutei using principal components analysis, with an application to larvae of *Strongylocentrotus droebachiensis*. In

Echinoderm Biology (ed. R. D. Burke, P. V. Mladenov, P. Lamber and R. L. Parsley), pp. 277–284. Rotterdam: A. A. Balkema.

HEBERT, P. D. N. (1978). The adaptive significance of cyclomorphosis in *Daphnia*: more possibilities. *Freshwater Biol.* **8**, 313–320.

HIDU, H. AND HASKIN, H. H. (1978). Swimming speeds of oyster larvae *Crassostrea virginica* in different salinities and temperatures. *Estuaries* **1**, 252–255.

HOCHACHKA, P. W. AND SOMERO, G. N. (1984). *Biochemical Adaptation*. Princeton: Princeton University Press.

JØRGENSEN, C. B. (1983). Fluid mechanical aspects of suspension feeding. *Mar. Ecol. Prog. Ser.* **11**, 89–103.

JØRGENSEN, C. B., FAMME, P., KRISTENSEN, H. S., LARSEN, P. S., MOHLENBERG, F. AND RIISGÅRD, H. U. (1986). The bivalve pump. *Mar. Ecol. Prog. Ser.* **34**, 69–77.

JØRGENSEN, C. B., LARSEN, P. S. AND RIISGÅRD, H. U. (1990). Effects of temperature on the mussel pump. *Mar. Ecol. Prog. Ser.* **64**, 89–97.

KOEHL, M. A. R. AND STRICKLER, J. R.(1981). Copepod feeding currents: Food capture at low Reynolds numbers. *Limnol. Oceanogr.* **26**, 1062–1073.

LABARBERA, M. (1984). Feeding currents and particle capture mechanisms in suspension feeding animals. *Am. Zool.* **24**, 71–84.

LEE, J. W. (1954). The effect of temperature on forward swimming speed in *Euglena* and *Chilomonas*. *Physiol. Zool.* **27**, 275–280.

LINLEY, J. R. (1986). Swimming behavior of the larva of *Culicoides variipennis* (Diptera: Ceratopogonidae) and its relationship to temperature and viscosity. *J. med. Ent.* **23**, 473–483.

MACHEMER, H. (1972). Ciliary activity and origin of metachrony in *Paramecium*: Effects of increased viscosity. *J. exp. Biol.* **57**, 239–259.

MACHEMER, H. (1974). Ciliary activity and metachronism in Protozoa. In *Cilia and Flagella* (ed. M. A. Sleigh), pp. 199–286. London: Academic Press.

MAEDA, K., IMAE, Y., SHIOI, J.-I. AND OOSAWA, F. (1976). Effect of temperature on the motility and chemotaxis of *Escherichia coli*. *J. Bacteriol.* **127**, 1039–1046.

MCCARTER, L., HILMEN, M. AND SILVERMAN, M. (1988). Flagellar dynamometer controls swarmer cell differentiation of *V.parahaemolyticus. Cell* **54**, 345–351.

MCEDWARD, L. R. (1984). Morphometric and metabolic analysis of the growth and form of an echinopluteus. *J. exp. mar. Biol. Ecol.* **82**, 259–287.

MCEDWARD, L. R. (1985). Effects of temperature on the body form, growth, electron transport system activity and development rate of an echinopluteus. *J. exp. mar. Biol. Ecol.* **93**, 169–181.

MILEIKOVSKY, S. A.(1973). Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. *Mar. Biol.* **23**, 11–17.

MITCHELL, J. G., MARTINEZ-ALONSO, M., LALUCAT, J., ESTEVE, I. AND BROWN, S. (1991). Velocity changes, long runs and reversals in the *Chromatium minus* swimming response. *J. Bacteriol.* **173**, 997–1003.

MOGAMI, Y., OOBAYASHI, C., YAMAGUCHI, T., OGISO, Y. AND BABA, S. A. (1988). Negative geotaxis in sea urchin larvae: a possible role of mechanoreception in the late stages of development. *J. exp. Biol.* **137**, 141–156.

MORTENSEN, T. (1921). *Studies of the Development and Larval Forms of Echinoderms.* Copenhagen: G. E. C. Gad.

OLSON, R. R. AND OLSON, M. H. (1989). Food limitation of planktotrophic larvae: does it control recruitment success? *A. Rev. ecol. Syst.* **20**, 225–247.

PAULAY, G., BORING, L. AND STRATHMANN, R. R. (1985). Food limited growth and development of larvae: experiments with natural sea water. *J. exp. mar. Biol. Ecol.* **93**, 1–10.

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

POWER, J. H. (1989). Sink or swim: growth dynamics and zooplankton hydromechanics. *Am. Nat.* **133**, 706–721.

PURCELL, E. M.(1977). Life at low Reynolds numbers. *Am. J. Phys.* **45**, 3–11.

REISWIG, H. M.(1975). The aquiferous systems of three marine Demospongiae. *J. Morph.* **145**, 493–502.

SCHMIDT-NIELSEN, K.(1990). *Animal Physiology: Adaptation and Environment*, 4th edition. Cambridge, UK: Cambridge University Press.

SHIMETA, J. AND JUMARS, P. A.(1991). Physical mechanisms and rates of particle capture by suspension feeders. *Oceanography and Marine Biology: An Annual Review* **29**, 191–257.

SLEIGH, M. A. (1956). Metachronism and frequency of beat in the peristomial cilia of Stentor. *J. exp. Biol.* **33**, 15–28.

SLEIGH, M. A. (1989). Adaptations of ciliary systems for the propulsion of water and mucus. *Comp. Biochem. Physiol.* **94**A, 359–364.

SLEIGH, M. A. AND BLAKE, J. R.(1977). Methods of ciliary propulsion and their size limitations. In *Scale Effects in Animal Locomotion* (ed. T. J. Pedley), pp. 243–256. London: Academic Press.

SMAYDA, T. J. (1970). The suspension and sinking of phytoplankton in the sea. *Oceanography and Marine Biology: An Annual Review* **8**, 354–414.

STEPHENS, R. E. (1973). A thermodynamic analysis of mitotic spindle equilibrium at active metaphase. *J. Cell Biol.* **57**, 133–147.

STRATHMANN, M. F. (1987). *Reproduction and Development of Marine Invertebrates of the Northern Coast.* Seattle: University of Washington Press.

STRATHMANN, R. R. (1971). The feeding behavior of planktotrophic larvae: mechanisms, regulation and rates of suspension-feeding. *J. exp. mar. Biol. Ecol.* **6**, 109–160.

STRATHMANN, R. R. (1985). Feeding and non-feeding larval development and life-history evolution in marine invertebrates. *A. Rev. ecol. Syst.* **16**, 339–361.

STRATHMANN, R. R., FENAUX, L. AND STRATHMANN, M. F. (1992). Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. *Evolution* **46**, 972–986.

STRICKLER, J. R. (1984). Sticky water: a selective force in copepod evolution. In *Trophic Interactions within Aquatic Ecosystems* (ed. D. G. Meyers and J. R. Strickler), pp. 187–242. Boulder, CO: Westview Press, Inc.

VOGEL, S.(1981). *Life in Moving Fluids.* Princeton, NJ: Princeton University Press.

WIESER, W.(1973). *Effects of Temperature on Ectothermic Organisms.* New York: Springer-Verlag.

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

WU, T. Y. (1977). Introduction to the scaling of aquatic animal locomotion. In *Scale Effects in Animal Locomotion* (ed. T. J. Pedley), pp. 203–232. London: Academic Press.

YONEDA, M. (1962). Force exerted by a single cilium of *Mytilus edulis.* II. Free motion. *J. exp. Biol.* **39**, 308–317.

ZAR, J. H. (1984). *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall, Inc.

ZARET, R. E. (1980). The animal and its viscous environment. In *The Evolution and Ecology of Zooplankton Communities* (ed. W. C. Kerfoot), pp. 3–9. Hanover, NH: University Press of New England.