PHYSICAL PROPERTIES OF GAMETES IN THREE SEA URCHIN SPECIES

FLORENCE I. M. THOMAS*

University of California at Berkeley, Berkeley, CA 94720, USA

Summary

Physical properties (density in $kg\,m^{-3}$, viscosity, sinking rates and dispersion rate) of the gametes and associated spawned materials were measured for three species of sea urchin, *Tripneustes gratilla*, *Echinometra mathaei* and *Colobocentrotus atratus*, from habitats that differ in wave exposure. The gametes of all three species are negatively buoyant, highly viscous and exhibit shear-thinning (a decrease in viscosity with increasing shear rate). Female gametes are more viscous than male gametes, and the viscosity of female gametes differs among the three species. The viscosity of female gametes is highest for *C. atratus*, the species from habitats most exposed to wave action. Within the species *T. gratilla*, viscosity of female gametes is higher in habitats exposed to wave action than in more protected habitats.

Evidence reported in this paper suggests that the shear-thinning of gametes may provide a performance advantage for these sea urchins. High viscosity of gametes at low shear rates may decrease gamete dispersal upon release and, under certain flow conditions, allow gametes to form strings and clumps on the surface of the urchin. Depending upon the morphology of the surface, these clumps or strings may be retained and fertilization may occur within these clumps or strings. Conversely, low viscosity of gametes at high shear rates decreases the power required to extrude gametes through the gonoduct during spawning.

Introduction

Fertilization success of free-spawning marine invertebrates

In many theories on the evolution of life-history patterns in marine invertebrate animals, selection for specific life-history traits acts through differential survival of larvae in the plankton, at settlement or after settlement rather than at fertilization (e.g. Vance, 1973*a*,*b*; Christiansen and Fenchel, 1979; Strathmann, 1985). Recent experimental and theoretical research on fertilization success in free-spawning marine invertebrate animals, however, has suggested that fertilization rates may be low and that these rates can be affected by factors such as water velocity in the habitat and aggregation of adults. Factors

that influence fertilization success, then, may also be important in the evolution of life-history traits. Free-spawning of gametes into the water column is a common mode of reproduction for many marine invertebrates; therefore, determining the success of fertilization for this mode of spawning is essential to development of appropriate life-history theories.

In the theoretical research on sperm dispersal in turbulent flow that predicts low fertilization success (Denny, 1988; Denny and Shibata, 1989), expected fertilization rates are calculated from estimates of sperm concentration in a plume downstream from a male releasing sperm at a constant rate. Low estimates of fertilization success result from rapid dispersion of gametes with distance from the spawning animal. Field experiments that have measured low fertilization rates include either measurement of fertilization success of eggs placed in some sort of array around a spawning male (Yund, 1990; Levitan, 1991; Levitan *et al.* 1992) or collection of water samples downstream of a spawning male and measurement of the fertilization effectiveness of these samples (Pennington, 1985).

Both the theoretical model and the field experiments assumed that gametes are freely dispersing and that they do not form large clumps or strings due to inter-gamete stickiness and/or viscosity of the material in which gametes are packaged. Evidence suggests, however, that this is not the case for all free-spawning marine invertebrates. Clumps or strings of gametes have been observed in some free-spawning invertebrates, such as an Antarctic limpet (Picken and Alan, 1983), sea cucumbers (McEuen, 1988), sea urchins (Woodward, 1940) and polychaetes (Thomas, 1994). Buoyancy of gametes can also differ between species (e.g. McEuen, 1988; Thomas, 1994). Therefore, it is possible that contact of sperm and eggs in free-spawning invertebrates occurs through several mechanisms, only one of which, the formation of gamete plumes, is measured or addressed by Denny and Shibata's model and by field experiments on invertebrate fertilization. For example, if strings or filaments of sperm form during spawning, these may stick to the surface of males, float intact in the water column, stick to females or attach to other features in the habitat. Such strings of sperm may, in effect, act as an egg filter or drift net; eggs captured on these filaments may be fertilized. Eggs fertilized in this way may be missed by the techniques that have been used to measure fertilization success in the field and are not considered by the model of Denny and Shibata (1989). Moreover, this model ignores aspects of local topography and adult morphology that could entrap released gametes, such as recirculating wakes or areas of reduced flow velocities found within groups of plants and animals (Monteith, 1973; Campbell, 1977; Eckman, 1983; Johnson, 1986). Thus, both the model and the field experiments mentioned previously may be underestimating fertilization success in free-spawning invertebrates.

Physical background

Since many free-spawning marine invertebrates are sessile, they depend on water motion for transport and for the mixing of gametes required for successful fertilization. The morphology of an organism (Chaimberlain and Graus, 1975; Koehl, 1977; Anderson and Charters, 1982) and its proximity to neighboring organisms (Eckman, 1979, 1983;

Johnson, 1986) influence the characteristics of the water flow it experiences. Furthermore, the height above the substratum at which gametes are released can influence their rate of mixing and transport in the water column. For example, if gametes are released close to the substratum, they may enter the benthic boundary layer (a region at the interface of the water and the substratum characterized by a velocity gradient). Flow velocity in this boundary layer is slow relative to the mainstream flow and rates of shearing are high (Okubo, 1980). Thus, rates of gamete transport may be slower and rates of gamete mixing faster in this boundary layer than in the ambient water flow because of the effects of shear dispersion, whereby particles in a diffusing cloud are spread by the shear within a velocity gradient (Okubo, 1980).

Physical properties, such as buoyancy and viscosity, of gametes may also affect rates of gamete mixing and transport in the water column. For example, the sinking rate of a substance determines where it is located in the water column and can, in turn, affect the velocity or turbulence of the water it encounters. The miscibility of a substance determines how easily it mixes with the surrounding water. The viscosity of gametes or the material in which they are packaged will determine how easily gametes can be removed from the surface of spawning animals or from other features in the habitat upon release, and the stickiness of gametes will determine whether they adhere to one another, forming clumps or strings. Furthermore, if gametes are released into the benthic boundary layer (where shear rates are high), the viscosity of the gametes will influence their rate of transport and mixing, since viscosity is a measure of the resistance of a material to shearing (Vogel, 1981).

Objectives of this study

In this study, four questions are addressed concerning the physical properties of the gametes (gametes together with any material co-released at spawning) of three species of sea urchin common in the Hawaiian islands: *Tripneustes gratilla* (Linné), *Echinometra mathaei* (Blainville) and *Colobocentrotus atratus* (Linnaeus). (1) How do the physical properties (density in $\mathrm{kg\,m}^{-3}$, sinking rate, dispersion rate and viscosity) of gametes vary among the three species? (2) How do these properties affect the transport of gametes from rough surfaces such as the surface of the animal? (3) How do these properties vary within a species for animals from habitats that experience different amounts of exposure to wave action? If the physical characteristics of gametes, such as viscosity, play a role in increasing fertilization success, it is possible that gametes of animals from these different habitats will exhibit different physical characteristics. For example, since viscosity resists shear at the surfaces of the animal, high viscosity may increase retention time of the gametes at the surface of the spawning animal or increase the formation of clumps or strings. Therefore, gametes from habitats more exposed to wave action may be more viscous than those from protected habitats. (4) How does the morphology of the spawning animal affect the rate of gamete transport from its surface?

I chose to address these questions using *T. gratilla*, *E. mathaei* and *C. atratus* since they are abundant in habitats that experience a range of exposures to wave action and they spawn relatively large quantities of material.

Materials and methods

Field sites and animals

Tripneustes gratilla is in the family Echinidae and is the largest of the three species used in this study. It has a rounded shape and relatively short spines (Table 1). This species is commonly found both in the shallow subtidal region of habitats exposed to wave action and in habitats that are protected from high wave action. *T. gratilla* were collected from three sites relatively protected from waves and from three relatively exposed sites. Protected sites included the following. (1) A protected reef bench on the north shore of Kappapa Island, located at the mouth of Kaneohe Bay, Oahu, Hawaii. This bench is behind a fringing rock reef and is not exposed to the full force of waves breaking on the outer edge of the island. (2) A rock bench on the Leeward side of Kappapa Island. (3) A reef located in Kaneohe Bay, Checker Reef. Exposed sites included three famous surfing sites where wave heights exceed 5 m during winter months (Wright, 1985) along the north shore of Oahu; (1) Laniakea Beach, (2) Chuns Reef and (3) Left-overs. Urchins from these exposed sites were all found at depths of 2.0–3.0 m below mean low water level, whereas urchins at protected sites were found at depths of 0.5–1.5 m below mean low water level. Urchins were sampled by hand; every third urchin encountered while snorkeling or walking a criss-cross pattern within the habitat was collected.

Echinometra mathaei is in the family Echinometridae. Like *T. gratilla*, it is common in both exposed and protected habitats. *E. mathaei* is round in shape, is smaller than *T. gratilla* and has relatively long spines (Table 1). Individuals were collected from the same protected sites as those from which *T. gratilla* were obtained (see above).

Colobocentrotus atratus is in the same family (Echinometridae) as *E. mathaei* and is the smallest of the three urchins. It has a flattened shape and short spines that are modified into flat plates (Table 1). This urchin commonly occurs in intertidal zones of very exposed habitats, where it clings tenaciously to rocks exposed to seasonally heavy wave action. *C. atratus* for this study were collected from three sites on the island of Oahu, Hawaii: (1) a rock reef at the outer edge of Kappapa Island; (2) an intertidal rock face below the Molokai-Lanai lookout on the south shore of Oahu and (3) an intertidal rock face below the Blow Hole lookout on the south shore of Oahu. These urchins were collected as encountered on the shore since they had to be grabbed between sets of breaking waves which did not allow time for careful selection of individuals. After

Table 1. *The mean test diameter and spine/test ratio (spine length/test diameter) for the three species of sea urchin* Tripneustes gratilla, Echinometra mathaei *and* Colobocentrotus atratus

	Test diameter (cm)	Spine/test ratio
Tripneustes gratilla	6.4±1.2	0.13±0.02
Echinometra mathaei	5.4±0.9	0.28±0.04
Colobocentrotus atratus	4.8±0.5	0.05±0.01

Values are mean ± S.D., N=10.

collection, the urchins were kept for 1–3 days in running sea water at the Hawaii Institute of Marine Biology, Coconut Island, Oahu.

Water velocities were measured at the rock bench on the outer edge of Kappapa Island by measuring, from video recordings, distances that particles in the water column moved with time on three separate days 12 October, 5 November and 7 November 1991. Particle movement was recorded with a video camera (Sony CCDV9) in an underwater housing (Ikelite) that was held on the substratum with lead weights. Estimates of $u*$, an index of turbulence that is related to the gradient in velocity within the boundary layer (Nowell and Jumars, 1984), for the water flow at this site were made using an empirical expression describing boundary layer flow in a rough pipe:

$$u* = u(c_f/2)^{1/2}, \tag{1}$$

where $(c_f/2)^{-1/2} = -5.1\log[6.9/Re + (k_s/14.8h)^{1.11}]$, $Re = \rho u D/\nu$, Re is the Reynolds number, ρ is the density of the water, ν is the kinematic viscosity of the water, c_f is the friction coefficient, $u*$ is the boundary shear velocity, u is the mean free-stream velocity, k_s is the roughness diameter (mean height of roughness elements in the habitat), and h is the water depth, $D = 4h$.

Application of this method to provide rough estimates of $u*$ for coral reef communities has been discussed by Bilger and Atkinson (1992). Roughness diameter in this site was estimated by measuring the height above the substratum of features in 20 haphazardly chosen locations within the habitat. For the Kaneohe bay sites, mean velocity was obtained from Bathen (1968) and estimates of $u*$ were obtained from Bilger and Atkinson (1992).

Collection of gametes

Urchins were induced to spawn by injecting them with $0.5\,\text{mol}\,l^{-1}$ KCl. Gametes were collected in a dry 1 ml syringe in air as they were released from the gonoducts of the urchins. Experiments were conducted immediately after collection.

Measurement of the physical characteristics of gametes

Viscosity

Viscosity of gametes from ten males and ten females of each species from each site was measured over a range of shear rates. These measurements were made with a cone and plate viscometer (Brookfield model DV-II) kept at a temperature of $26\,°\text{C}$ using a constant temperature bath (Haake FK). Each viscosity measurement was made on 0.5 ml of gametes, the volume required by this model of viscometer. In each case, measurements were begun at a low shear rate ($0.6\,\text{s}^{-1}$) and shear rate was then increased in steps to 1.2, 3.0, 6.0, 12.0, 23.9 and $59.8\,\text{s}^{-1}$. Each shear rate was maintained for 20 s to ensure that the viscosity measurement had reached a steady value. In addition, for gametes with lower viscosity, an additional shear rate of $119.8\,\text{s}^{-1}$ was used. After obtaining the highest shear rate, the procedure was reversed by measuring the viscosity from the highest to the lowest shear rates.

Five female and four male *E. mathaei* spawned spontaneously in the laboratory and gametes were collected from these animals without KCl injection. To determine whether

the induction of spawning affected the measured viscosities of gametes, the viscosities of these naturally spawned gametes were compared with those from induced spawning for animals from the same site.

To determine whether the viscosity of the gametes fits a common relationship for non-Newtonian fluids, a power law was used of the form:

$$\text{Shear stress} = k(\mathrm{d}u/\mathrm{d}r)^n, \tag{2}$$

where shear stress is the applied force per unit area, $\mathrm{d}u/\mathrm{d}r$ is the shear rate (i.e. the rate at which the force is applied), u is the velocity of the fluid, r is the distance from the wall, k is a proportionality constant and n is a power coefficient. A value for n of less than 1 indicates that the fluid is shear-thinning (the viscosity of the material decreases with increasing shear) (Hughes and Brighton, 1967). The proportionality constant k describes the consistency of the fluid. Higher values of k indicate a greater apparent viscosity for the fluid over all shear rates.

To ascertain the field conditions that would produce shear rates in the range of greatest viscosity differences, shear rates at which greatest differences among the three species occurred were determined from plots of viscosity *versus* shear rate. Equation 2 was used to determine shear stresses at these critical shear rates with the assumption that the shear stresses experienced by the gametes would equal the shear stress of the surrounding water. The following relationship was then used to determine the value of u^* required to exceed this shear stress in the field:

$$u^* = (Re_\mathrm{s}/\rho)^{1/2}, \tag{3}$$

where u^* is the shear velocity, Re_s is the Reynolds shear stress (shear due to velocity fluctuations) and ρ is the fluid density.

The effect of temperature on the viscosity of the gametes for *T. gratilla* and *E. mathaei* from the Kaneohe Bay habitats was also examined. The viscosity of the gametes from five individuals of each sex from each site was measured, as described above, with the viscometer held at $10\,°\mathrm{C}$. These viscosities were compared with those measured for the gametes at $26\,°\mathrm{C}$.

To determine whether these gametes exhibit thixotropy (either increases or decreases in viscosity over time), the viscometer was set at a shear rate of $1.2\,\mathrm{s}^{-1}$ and the viscosity of three individuals of each sex and species was recorded every minute for 15 min. This was repeated for a shear rate of $59.8\,\mathrm{s}^{-1}$. In addition, the viscosity measured as shear rate was increased was compared with the viscosity measured as shear rate was decreased for gametes of both males and females of each species.

To ensure that all gametes used in these studies were mature, the fertilization success of the eggs was determined. Ten males and 10 females of each urchin species were tested. A random sample of the urchins used for the measurements of the viscosities of gametes was taken for measurements of fertilization success. Fertilization success was determined by adding 1 ml of urchin eggs to sperm solutions within a concentration range known to produce a high fertilization success for sea urchins (1 ml of sperm per 100 ml of sea water) (Pennington, 1985). This mixture of gametes was kept at a constant temperature of $22\,°\mathrm{C}$ for 15 min and was swirled every 5 min to keep the gametes mixed within the water

sample. A sample of eggs from this solution was then removed using a pipette, the number of eggs with and without fertilization membranes was counted and the percentage fertilized was calculated.

Density, sinking and dispersion rates

The density of gametes of nine male and nine female urchins of each species were measured. A known volume of gametes was weighed to the nearest 0.1 mg on a Metler AJ100 balance and their density was calculated. The sinking rates of gametes in still water were measured by releasing a 0.1 ml sample of gametes from a height of 0.2 m above the bottom of a large (0.3 m diameter) cylindrical glass container and recording the time required for this clump of gametes to sink a distance of 0.1 m. Sinking rates were measured for nine males and nine females of each species.

To determine rates of dispersion of gametes, the time taken for a 0.1 ml clump of gametes to dilute in a 0.3 m diameter glass container filled with sea water kept at 26 °C within a constant temperature bath (Haake FK) was measured. Gametes were released from a height of 0.2 m above the bottom of the container through a hollow metal tube. The water in the container was stirred gently with a flat plastic paddle from the surface of the water at a constant rate of 6 revs s^{-1}. The time required to break up the entire clump of gametes was measured for nine females and nine males of each species.

Consequences of high gamete viscosity and shear thinning

Gamete extrusion from gonopores

To determine how the viscosity and non-Newtonian behaviour of gametes affects their extrusion from gonopores during spawning, the power required to force gametes through urchin gonoducts was calculated using the Hagen–Poiseuille equation for flow through circular pipes (see Vogel, 1981, and equation 2) as follows:

$$P = \Delta p \times Q, \tag{4}$$

where Δp is the pressure drop across the length of gonoduct, Q is the rate of gamete release and P is the power required to extrude the gametes through the duct. The pressure drop across a length of gonoduct (Δp) was derived as follows:

$$\pi r \Delta p = -2\pi l k (\mathrm{d}u/\mathrm{d}r)^n, \tag{5}$$

rearranging, solving for $u(r)$, then integrating and assuming the no-slip condition, $u(0)=0$, yields:

$$u(r) = -(\Delta p/2lk)^{1/n}[n/(n+1)] \times [a^{(n+1)/n} - r^{(n+1)/n}]. \tag{6}$$

Further, $Q=2\pi r[u(r)]\mathrm{d}r$; therefore, substituting for $u(r)$ and rearranging for Δp yields the pressure drop (Δp) as follows:

$$\Delta p = \langle Q/\{2\pi[n/(n+1)] \times \{1/2 - [n/(3n+1)]\} \times a^{(3n+1)/n}\}^n \times 2lk, \tag{7}$$

where r is the distance from the wall, u is the fluid velocity, l is the length of gonoduct, k is the proportionality constant from equation 2, n is the power coefficient from equation 2, and a is the diameter of the gonoduct.

This calculated power for gametes exhibiting non-Newtonian shear-thinning behaviour (a decrease in viscosity with shear rate) was compared with the power required to extrude a Newtonian fluid with a constant viscosity of either 10 Pa (the highest mean value measured for the gametes at low shear rates) or 2 Pa (the lowest mean value for the gametes at low shear rates). Measured values of the proportionality constant (k) and power coefficient (n) from equation 2 were used (see Table 3). The same dimensions for the gonoduct were assumed for all three species (length 2 cm, diameter 0.5 mm, which is within the range common for sea urchins; Emlet, 1989). The rates of gamete extrusion (Q) were varied from 0.1×10^{-10} ml s^{-1} to 10×10^{-10} ml s^{-1}.

Clearance rates of gametes and effects of urchin morphology

The time that clumps of gametes remain attached to a rough surface was measured under different mainstream water velocities in a unidirectional flow tank of length 1.2 m, width 0.25 m and depth 0.25 m (Vogel and Labarbera, 1978). Conditions in the flow tank were set so that they were within the range experienced by the animals from the Kaneohe Bay sites (Table 2). Mainstream water velocities were set at 0.06 m s^{-1} and 0.28 m s^{-1}. Shear velocities in the tank were controlled at these two mainstream flow velocities by placing a collimator, made from various lengths of straws at different heights, in the tank. The shear velocity in the tank was determined by measuring the velocity of the water flow at four heights (2, 4, 6 and 10 cm) above the substratum. Shear velocity (u^*) was then estimated using a logarithmic regression (Campbell, 1977). To measure velocities at different heights within the tank, video recordings made using a SONY CCDV9 Cam-Corder of the movement of particles were analyzed. Shear velocity (u^*) in the tank was 4×10^{-3} m s^{-1} ($u=0.05+0.002\ln x$, where u is water velocity and x is height above the substratum, $r^2=0.989$) at the lower velocity and 3×10^{-2} m s^{-1} ($u=0.20+0.012\ln x$, $r^2=0.978$) at the higher velocity.

To determine whether gametes can remain attached to a rough surface, the time required to clear a 0.1 ml sample of gametes from sandpaper was measured by placing a sample of either male or female gametes onto no. 80 wet–dry sandpaper glued to a weighted piece of wood covering the bottom of the flow tank. The dispersal of these gametes was recorded on video tape and frame-by-frame analysis of the recordings enabled the measurement of the time required to clear the clump of gametes from the

Table 2. *Range of flow characteristics for two sites within Kaneohe Bay and one site on Kappapa Island*

	Velocity range (m s^{-1})	u^* range (m s^{-1})
Reefs in Kaneohe Bay	0.03–0.09	0.004–0.006
Rock Bench, Kappapa Island	0.03–0.6	0.002–0.05

The flow velocities for the sites within Kaneohe Bay are from Bathen (1968) and the boundary shear velocities (u^*) for these sites are from Bilger and Atkinson (1992).

Flow velocities for the reef bench site on Kappapa Island were measured from video tape analysis of particle motion at the site. Estimates of u^* for the Kappapa Island site were calculated using equation 1.

surface of the sandpaper. Clearance rates for nine females and nine males of each species under the two flow regimes described above were determined. Clearance rates of nine 0.1 ml samples of fluorescein dye ($4\,g\,l^{-1}$) were also obtained.

The mainstream flow velocity required to stretch the samples of gametes and to break off clumps from the samples was measured by placing samples of gametes on sandpaper as described above. These samples were placed in the flow tank at a mainstream velocity of $0.06\,m\,s^{-1}$. After 5 s the water velocity was increased to $0.13\,m\,s^{-1}$, then to $0.20\,m\,s^{-1}$ and finally to $0.28\,m\,s^{-1}$. The effects of increasing water velocity on the gametes were recorded on video tape. These recordings were then analyzed to determine the velocities at which gametes began (1) to stretch along the surface of the sandpaper and (2) to break into smaller clumps. Clumps of gametes were also observed at a water flow velocity $0.06\,m\,s^{-1}$ for 30 s to ensure that time alone was not responsible for changes in the behaviour of the gamete samples at different flow velocities.

To assess the possible role of urchin spine morphology on the clearance of gametes from the surface of the urchins, 0.5 ml samples of gametes were placed on the surface of an urchin and the dispersal of the gametes from the surface was recorded on video tape. Analysis of these recordings gave measurements of the time required for the gametes to be cleared (no gametes were visible) from the surface of the urchins. This procedure was repeated for five individuals of each sex for each species. The time required for a 0.5 ml sample of fluorescein dye to be cleared from the surface of each urchin was also determined. Clearance rates were measured under the two flow regimes described above. Results obtained for each species and for fluorescein dye were compared.

Results

Mean flow velocities and boundary shear velocities for protected habitats are summarized in Table 2. No attempt was made to characterize water flow conditions in exposed habitats (see Denny, 1988, for discussions of water flow characteristics in wave swept habitats).

Measurements of the physical properties of gametes

Viscosity

In each case gametes exhibited shear-thinning (Fig. 1), that is, the viscosity of the gametes decreased with increasing rates of shear. This non-Newtonian behaviour was apparent in the power coefficients (*n*) calculated for gametes of each species. In each case, the coefficient was less than one, indicating shear-thinning (Table 3).

The female gametes of all three species were significantly more viscous at each shear rate than were those of males (Fig. 1). The viscosities of male gametes did not differ significantly among the three species; however, the viscosities of female gametes differed significantly from those of the other species at each shear rate (Fig. 1). A *post hoc* test (Tukey's) indicated that the effect of species on viscosity of female gametes was significant for each pair of species ($P<0.01$) except at the highest shear rate, where the viscosities of female gametes of *E. mathaei* and *T. gratilla* did not differ significantly from one another. These relationships among viscosities were reflected in the
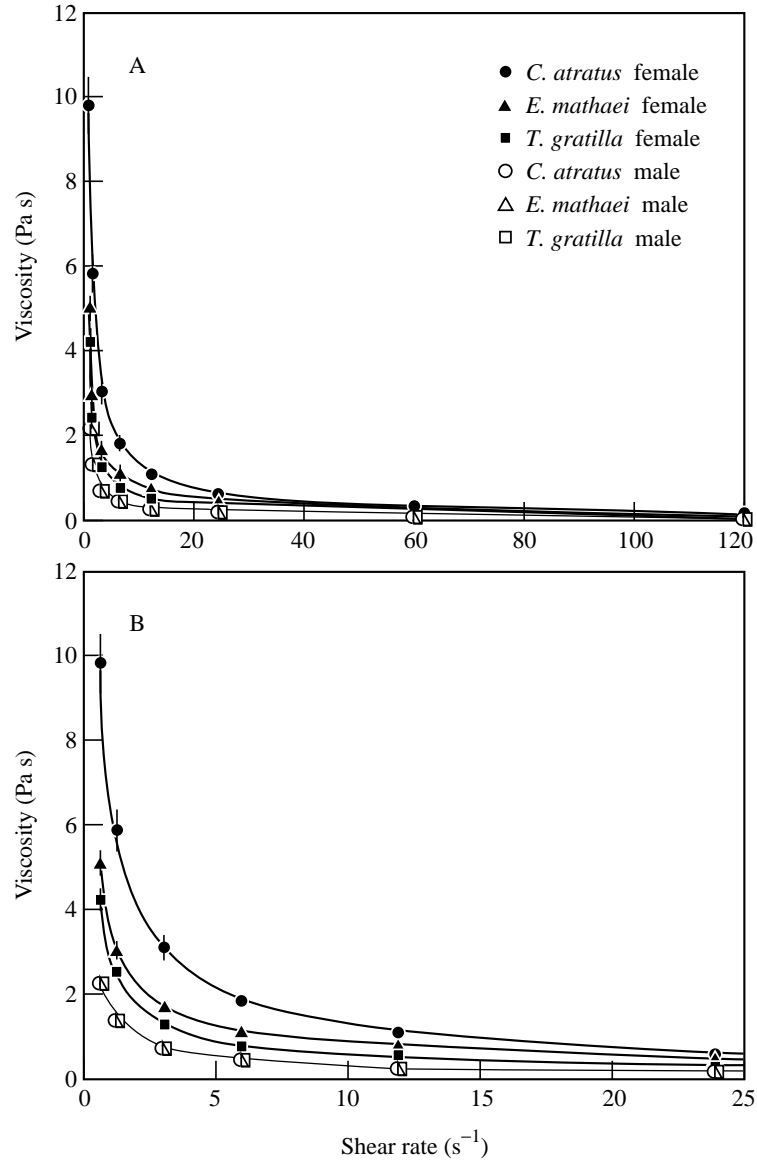
Fig. 1. Mean and standard error (bars) of the viscosity of gametes measured at various shear rates. Each point represents the mean of 30 (for *Echinometra mathaei* and *Colobocentrotus atratus*) or 60 (for *Tripneustes gratilla*) measurements. (A) The entire viscosity range tested; (B) an enlargement of the region below a shear rate of $25\,\mathrm{s^{-1}}$. Male gametes did not differ significantly among the three species at any shear rate (ANOVA, d.f.=2, $F<1.0$, $P>0.05$), while those of females did (ANOVA, d.f.=2, $F>60$, $P<0.01$). For each species, female gametes were significantly more viscous than male gametes (ANOVA, d.f.=1, $F>5.1$, $P<0.01$).

Table 3. *Mean values of density, sinking rate and dispersion time for male and female gametes of three sea urchin species (*T. gratilla*, *E. mathaei *and* C. atratus*)*

		$10^{-3} \times$ density (kg m^{-3})	Sinking rate (m s^{-1})	Dispersion time (s)	n	k
Tripneustes gratilla	Female	1.03±0.06	0.25±0.07	68±28	0.36	3.0
	Male	1.07±0.04	0.26±0.08	90±33	0.35	1.5
Echinometra mathaei	Female	1.08±0.17	0.30±0.06	71±23	0.36	3.6
	Male	1.05±0.12	0.23±0.06	90±38	0.37	1.5
Colobocentrotus atratus	Female	1.05±0.22	0.23±0.06	107±64	0.24	6.8
	Male	1.02±0.17	0.25±0.08	91±37	0.38	1.5

The power coefficient (n) and proportionality constant (k) are calculated for each sex and species using equation 2 ($n=30$ for *E. mathaei* and *C. atratus* and $n=60$ for *T. gratilla*).

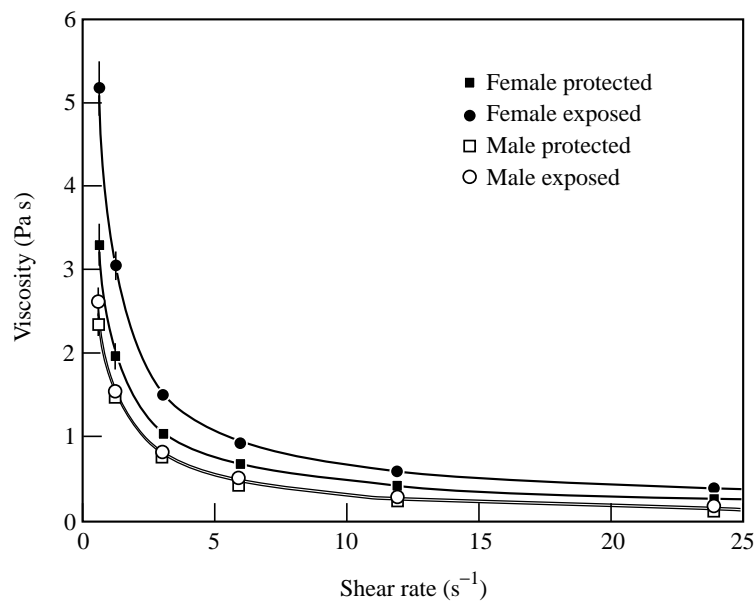Values are means ± S.D., $N=9$.

Fig. 2. Mean and standard error (bars) of the viscosity of gametes of *Tripneustes gratilla* measured at various shear rates. Each point represents the mean of 30 measurements. The viscosity of female gametes differed significantly with degree of exposure (ANOVA, d.f.=1, $F>5$, $P<0.01$). Values for the male gametes, however, did not differ significantly from each other at any shear rate (ANOVA, d.f.=1, $F<0.9$, $P>0.1$).

proportionality constant (k) calculated using equation 2 for each species (Table 3). *C. atratus* had the highest value of k, followed by *E. mathaei* and, finally, by *T. gratilla*.

The viscosities of female gametes of *T. gratilla* from the North Shore (exposed) sites were higher than viscosities measured for these gametes at more protected sites
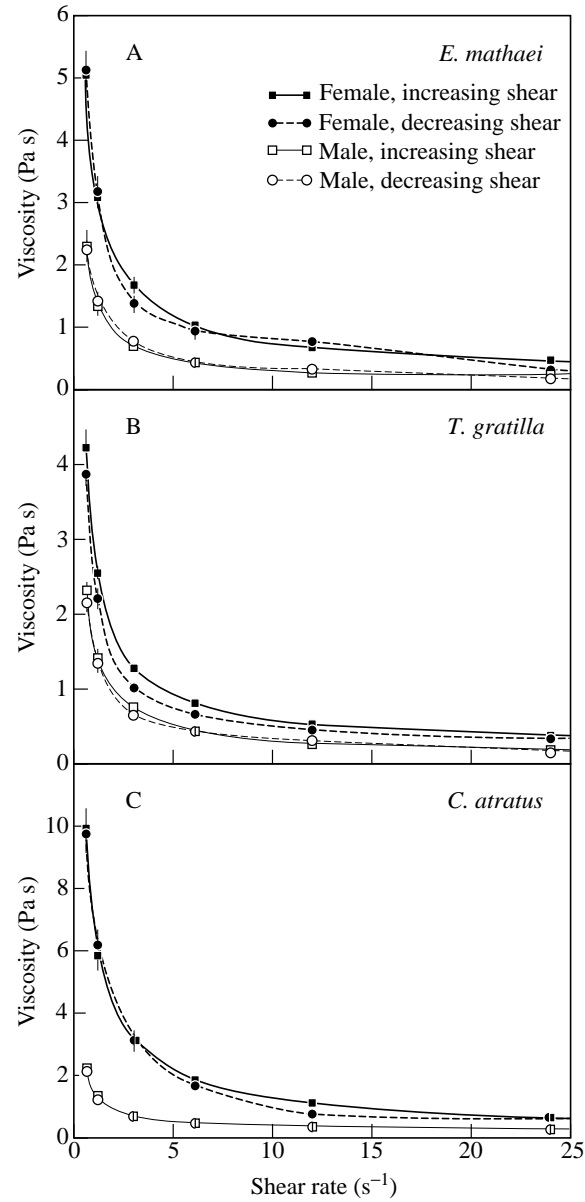
Fig. 3. Mean and standard error (bars) of viscosity for the gametes of three species of sea urchin, *Echinometra mathaei, Tripneustes gratilla* and *Colobocentrotus atratus*, measured at various shear rates. Each point represents the mean of the values measured for that species and sex (*N*=60 for *T. gratilla* and 30 for *C. atratus* and *E. mathaei*). There was no significant difference in viscosity at any shear rate between measurements taken while shear rate increased and those taken while shear rate decreased for any species or sex (ANOVA, d.f.=1, *F*<0.7, *P*>0.1).

(Fig. 2). None of the gametes display thixotropic characteristics (changes in viscosity with duration of shear). Moreover, viscosities measured while shear rates were increased did not differ significantly from those measured as the shear rate was decreased (Fig. 3).

The viscosities of the naturally spawned gametes of *E. mathaei* from Checker Reef did not differ significantly from the viscosities of the gametes from induced spawning at this site at any shear rate (ANOVA, d.f.=1, $F<2.9$ for males and $F<0.8$ for females, $P>0.17$). The viscosities of gametes measured at 10 °C did not differ significantly from viscosities measured at 26 °C for either *E. mathaei* or *T. gratilla* at any shear rate (Fig. 4).
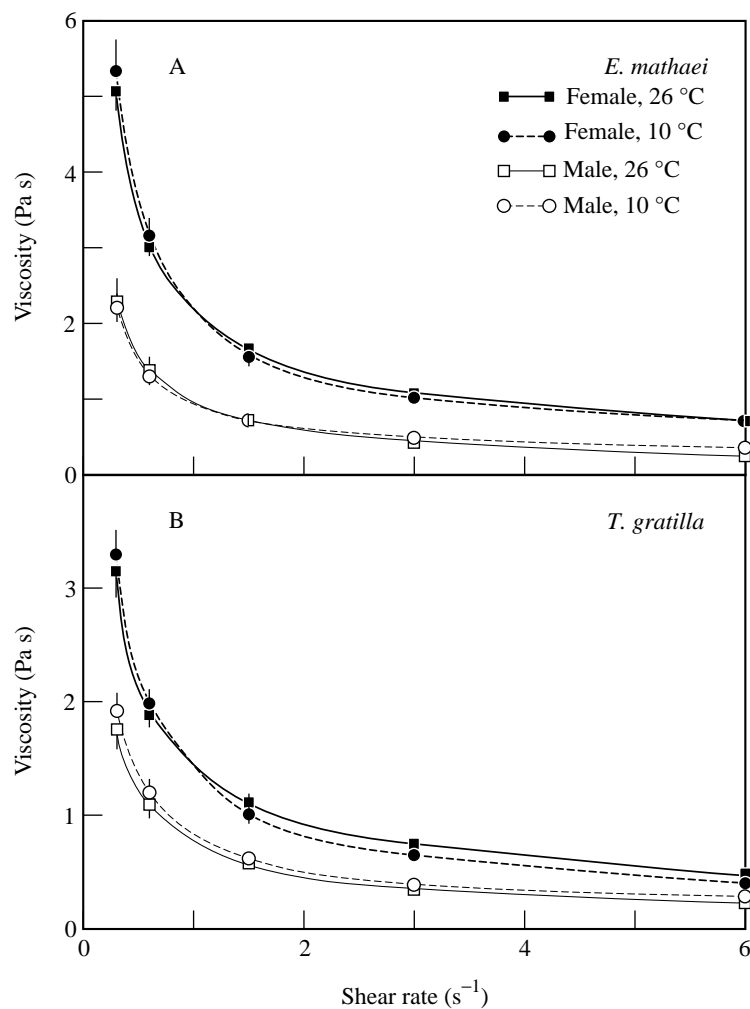

Fig. 4. Mean and standard deviation (S.D.) of viscosity of *Echinometra mathaei* and *Tripneustes gratilla* gametes measured at 26 °C (*N*=30) and 10 °C (*N*=15). There were no significant differences in the viscosities measured at the two temperatures at any shear rate (ANOVA, d.f.=1, $F<2$, $P>0.1$).

Rapid changes in the viscosities of gametes, and the greatest differences in viscosity among the species and sexes, occurred at shear rates below $6\,\mathrm{s}^{-1}$ (Fig. 1). Shear velocities (u^*, calculated from equation 3 for a Reynolds shear stress that would produce a shear rate of $6\,\mathrm{s}^{-1}$) above $0.08\,\mathrm{m\,s}^{-1}$ for female gametes and $0.05\,\mathrm{m\,s}^{-1}$ for male gametes are required to produce shear rates in excess of $6\,\mathrm{s}^{-1}$ in *T. gratilla* and *E. mathaei*. For *C. atratus* (the species from exposed habitats) a shear velocity greater than $0.10\,\mathrm{m\,s}^{-1}$ is necessary to exceed shear rates of $6\,\mathrm{s}^{-1}$ in female gametes while a shear velocity of $0.05\,\mathrm{m\,s}^{-1}$ is sufficient to produce a similar shear rate in male gametes.

Eggs used in these experiments were fertilizable, indicating that they were mature. The mean percentage fertilized in *T. gratilla* was 98±2, in *E. mathaei* was 95±5, and in *C. atratus* was 99±1 (values are mean ± standard deviation for N=10 samples).

Density, sinking and dispersion rates

Gametes of males and females of these three sea urchin species were more dense than sea water (Table 3). There was no significant difference in sinking rates of gametes either among species (ANOVA, d.f.=2, N=60, F=0.5 for females and F=1.6 for males, P>0.2) or between sexes (ANOVA, d.f.=2, N=40, F=0.07 for *E. mathaei*, F=0.5 for *T. gratilla* and F=0.01 for *C. atratus*, P>0.4). This is consistent with the similar densities ($\mathrm{kg\,m}^{-3}$) measured for the gametes (Table 3). There was a significant difference among the three species in dispersion rates of female gametes (ANOVA, d.f.=2, N=60, F=5.7, P=0.005). A *post hoc* test (Tukey's) indicates that dispersion rates of gametes of *T. gratilla* and *E. mathaei* do not differ significantly (P=0.99); however, female gametes of both these species were dispersed significantly faster than those of *C. atratus* (Table 3). There was no significant difference in dispersion rates in male gametes in the three species (ANOVA, d.f.=2, N=60, F=0.009, P>99) (Table 3). In *E. mathaei* (ANOVA, d.f.=1, F=5.8, P=0.02) and *T. gratilla* (ANOVA, d.f.=1, F=5.3, P=0.27), eggs were dispersed significantly more rapidly than sperm; however, in *C. atratus* there was no significant difference in the dispersion rates of eggs and sperm (ANOVA, d.f.=1, F=0.82, N=40, P=0.37) (Table 3).

Consequences of high viscosity and shear thinning

Gamete extrusion from gonopores

Shear-thinning of gametes greatly reduces the power required to extrude them from gonopores at release rates (Q) greater than $7\times10^{-11}\,\mathrm{m}^3\,\mathrm{s}^{-1}$ (Fig. 5). Furthermore, as Q increases, the difference between the power required to extrude a Newtonian fluid and that required to extrude these non-Newtonian fluids increases.

Clearance rates of gametes and effects of urchin morphology

Gametes of the three species of urchins tested are sticky and remain attached to rough surfaces. This resulted in slower clearance rates for gametes than for fluorescein dye from both sandpaper and from whole urchins (Figs 6 and 7). There was no significant difference among the three species in the clearance rates of male gametes from sandpaper at either flow velocity. However, at the higher velocity ($0.28\,\mathrm{m\,s}^{-1}$), the clearance rates
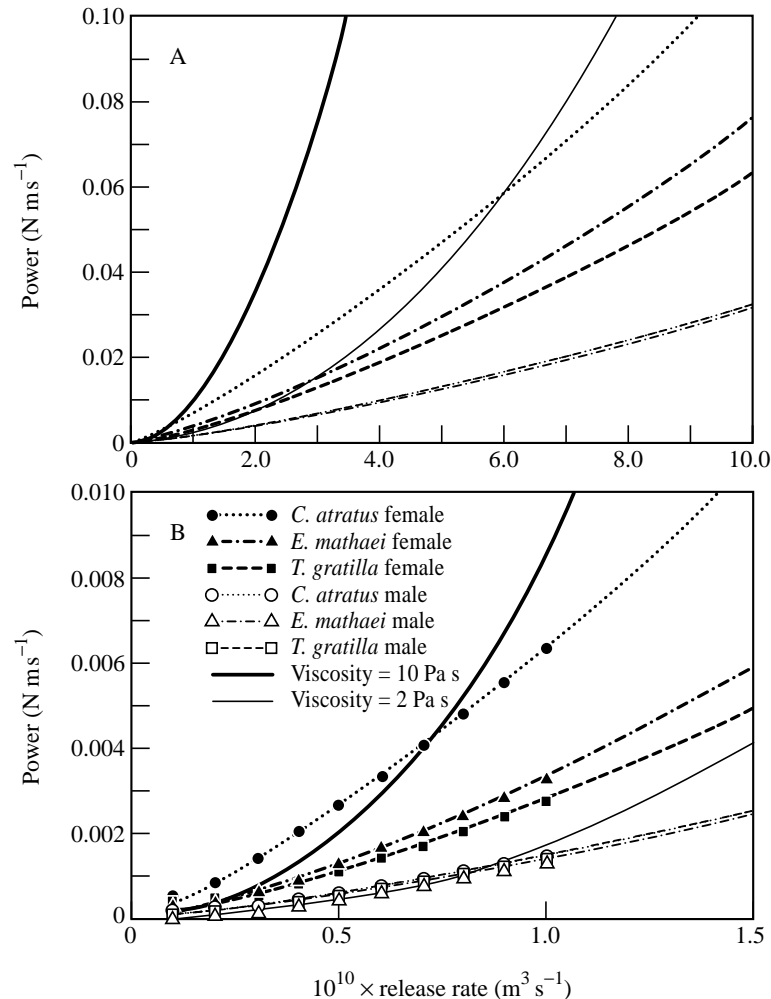
Fig. 5. (A) Power required to extrude gametes from the gonopores of males and females of *Tripneustes gratilla, Echinometra mathaei* and *Colobocentrotus atratus* for gamete release rates ranging from 0.1×10^{-10} to $10 \times 10^{-10} \, \text{m}^3 \, \text{s}^{-1}$. Solid lines indicate the power required to extrude a Newtonian fluid, one not exhibiting shear-thinning, that has a viscosity of $2 \, \text{Pa s}$ or $10 \, \text{Pa s}$. (B) An enlargement of A for release rates (Q) ranging from 0.1×10^{-10} to $1.5 \times 10^{-10} \, \text{m}^3 \, \text{s}^{-1}$.

for female gametes differed significantly. *T. gratilla* had the fastest clearance rate while *C. atratus* had the slowest. Male gametes of all three species stick to sandpaper longer than do female gametes (Fig. 6).

The morphology of urchins had a significant effect on rates of transport of water and gametes from urchin surfaces (Fig. 7). Fluorescein dye was retained significantly longer at surfaces of *E. mathaei* than at surfaces of either *T. gratilla* or *C. atratus*. This effect of morphology was reflected in differences in clearing rates of sperm from sea urchin tests
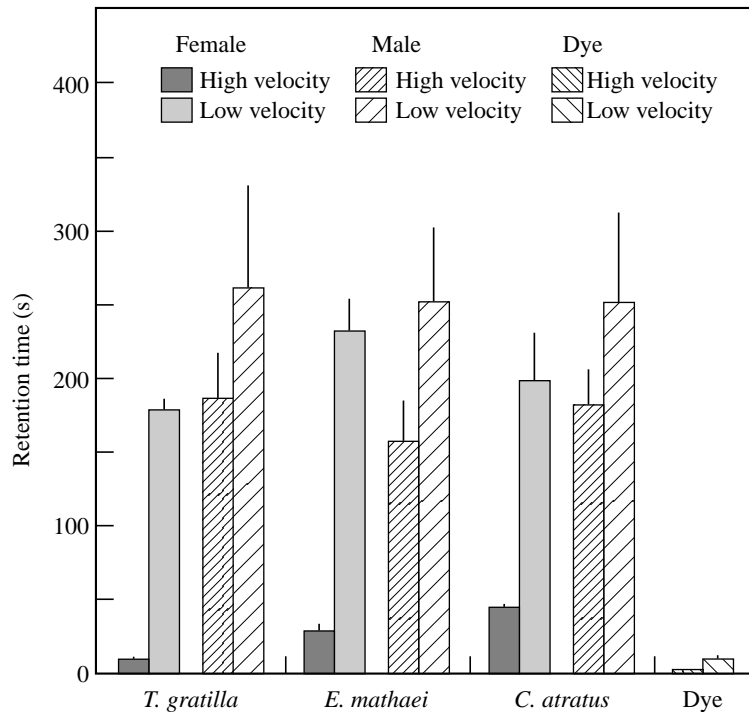
Fig. 6. Mean (N=9) and standard error (bars) of the retention time of gametes and dye on no. 80 wet–dry sandpaper at a low (0.06 m s^{-1}, u^*=4×10^{-3} m s^{-1}) and high (0.28 m s^{-1}, u^*=3×10^{-2} m s^{-1}) velocity. At the higher velocity, the retention rates of the female gametes differed significantly among the three species (ANOVA, d.f.=2, N=27, F=28.6, P<0.01). A *post-hoc* test (Tukey's) indicated that the rate for each species is significantly different from that for each of the other species (P<0.01). The female gametes show no significant differences among the three species at the slower water velocity (ANOVA, d.f.=2, N=27, F=1.4, P>0.3). There was no significant difference in retention times of the sperm among the three species at either flow velocity (ANOVA, d.f.=2, N=27, F=0.36 for the higher velocity and F=0.02 for the slower velocity, P>0.5). In all cases, gametes had a slower clearance rate than dye (ANOVA, d.f.=1, F>5, P<0.01).

among the three species (Fig. 7). The effect of morphology was also apparent in the comparison of clearance rates for male gametes from sandpaper compared with clearance rates from urchin tests (Figs 6 and 7). For *E. mathaei* and *T. gratilla*, clearance rates from urchins were slower than clearance rates from sandpaper at the higher velocity. However, for *C. atratus* (the species with the lowest ratio of spine length to test diameter, Table 1), clearance rates from tests and sandpaper did not differ significantly.

Observations of spawning in the flow tank indicated that both plumes and clumps of gametes form. Gametes formed a reservoir on the surfaces of the animals at velocities below 0.13 m s^{-1}. When the velocity was increased, after the formation of the reservoir, clumps of gametes were sloughed off the reservoir at velocities above 0.14 m s^{-1}. In addition, sperm strings 3–4 cm long formed on the spines of both *T. gratilla* and
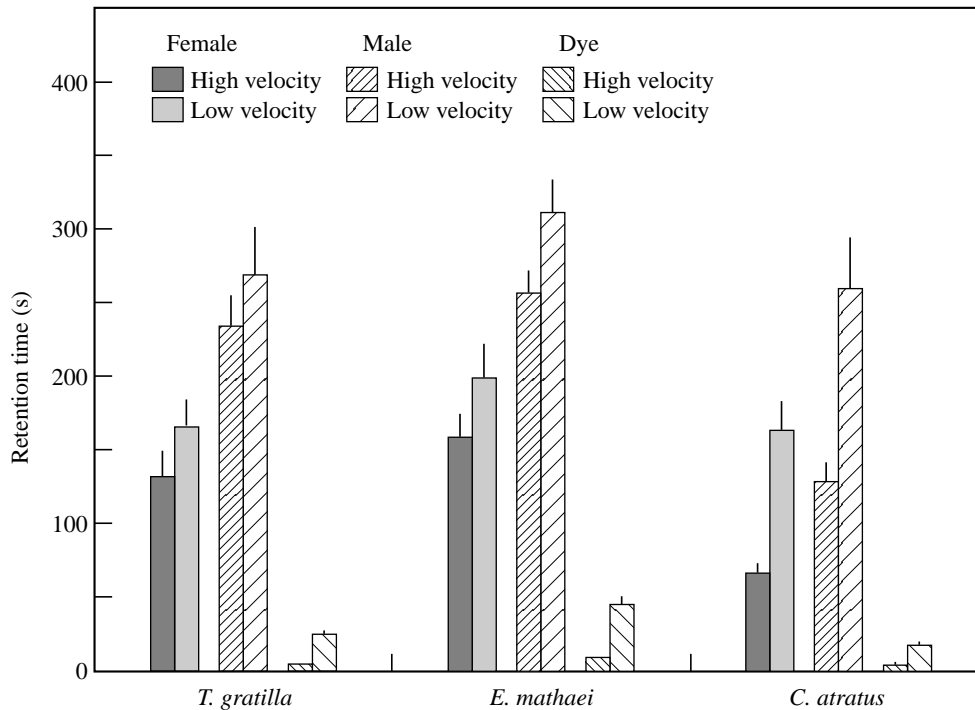
Fig. 7. Mean (N=9) and standard error (bars) of the retention time of gametes and dye on urchin tests at a low (0.06 m s^{-1}, u*=4×10^{-3} m s^{-1}) and high (0.28 m s^{-1}, u*=3×10^{-2} m s^{-1}) velocity. In each case, the retention time of gametes on the tests was longer than the retention of dye (ANOVA, d.f.=1, F>4, P<0.01). There was no significant difference in the retention time of the male (ANOVA, d.f.=2, F=0.97, P>0.4) or female (ANOVA, d.f.=2, F=1.0, P>0.3) gametes among the three species at low velocity; however, at the higher velocity, there was a significant effect of urchin morphology on the retention time of both the male (ANOVA, d.f.=2, F=17.7, P<0.03) and female (ANOVA, d.f.=2, F=13.8, P<0.01) gametes. A *post-hoc* test (Tukey's) indicates that the retention times of *C. atratus* female and male gametes (P<0.01 in each case) were lower than those for the other two species.

E. mathaei. This is consistent with observations that clumps of sperm in the sandpaper experiments remained intact at velocities below 0.13 m s^{-1}, but began to stretch at a velocity of 0.13 m s^{-1} and to break off at velocities in excess of 0.20 m s^{-1}. Clumps of eggs never stretched along the surface of the sandpaper. Individual eggs were removed singly from the surface of the clump by the moving water until flow velocities in excess of 0.20 m s^{-1} were reached. At this time, smaller clumps began to break free from the original clump.

When animals spawned at flow velocities that never dropped below 0.14 m s^{-1}, plumes of gametes formed. Under these conditions, clumps were rarely formed and those that were formed were less than 5 mm long. However, short (<10 mm long) sperm strings were seen.

Discussion

Results from this study indicate that gametes of all three sea urchin species tested are highly viscous and display shear-thinning behaviour. The gametes are also negatively buoyant and have lower dispersion rates than freely diffusing fluorescein dye. Furthermore, the gametes resist being dislodged from the substratum. This results in slow clearance rates of gametes from urchin tests and from other rough surfaces. In addition, gamete clumps and, in the case of males, strings form during spawning if the urchin releases gametes in habitats where mainstream velocities fall below $0.13\,\mathrm{m\,s^{-1}}$. Clump formation and resistance to clearance are probably the result of the high viscosities of the gametes at low shear rates. Thus, the viscosity of gametes may act to decrease the rapid spread of gametes under some bulk flow conditions.

Effects on fertilization

High viscosities of gametes at low shear rates probably contribute to the formation of clumps and strings. In situations where clumping of gametes occurs, it is probable that fertilization can take place while the gametes are held in clumps or strings. For example, eggs in clumps may become fertilized by sperm in the water column before leaving the surface of the spawning female. In addition, clumps of sperm may be caught on the spines of nearby females. Sperm dispersing from these clumps may fertilize eggs as they are released by the female. Sperm strings may also act as an egg filter, whereby eggs carried in the water are caught on the filament and fertilized. In addition, gamete clumps may sink and become trapped in areas between roughness elements both in the habitat and at the surface of the urchins. Gametes trapped in this way may remain in high concentrations, allowing fertilization to occur. Formation of gamete strings has been reported for other invertebrates, such as limpets (Picken and Alan, 1983), polychaetes (Thomas, 1994) and sea cucumbers (McEuen, 1988). Gamete strings have also been observed in red algae (Fetter and Neushul, 1981) and sea grasses (Cox, 1983, 1985). Cox suggests that these strings increase the probability of gamete encounters and may be an adaptation to aquatic life.

If considerable rates of fertilization occur in clumps and strings of gametes, as suggested here, theoretical models such as Denny and Shibata's model (Denny, 1988; Denny and Shibata, 1989) and field experiments (e.g. Pennington, 1985; Yund, 1990; Levitan, 1991; Levitan, *et al.* 1992) may underestimate fertilization rates for free-spawning invertebrates. In addition, these studies ignore the effects of local topography and animal morphology that may also decrease the rapid spread of gametes in marine habitats.

The idea that fertilization is facilitated by the formation of clumps or strings is consistent with the fact that some sea urchin species have sperm of more than one morphology (Eckelbarger *et al.* 1983). These researchers found that an abyssal urchin *Phrissocystis multispina* produces two types of sperm, one with a typical sperm morphology and the other with a bipolar tail. Sperm with bipolar tails may be more apt to become entangled and form clumps. Different sperm morphologies within and between species have also been observed in other invertebrates, such as polychaetes (Franzen and

Rice, 1988). In addition, Woodward, (1940) observed the formation of sperm clumps in the echinoid *Goniobasisi laqueata* during spawning and suggested that the formation of these clumps may be the function of an atypical sperm morphology.

In the three species of sea urchins observed in the present study, gamete clumps and strings only formed when the water velocity was held at $0.13\,\mathrm{m\,s^{-1}}$ for a short period (about 0.5 s). Therefore, the plume model may be accurate for these urchins under most flow conditions. However, before the assumption of fertilization in a plume is adopted for a species, the tendency of its gametes to form clumps or strings under the flow conditions it encounters in the field should be assessed. Furthermore, the effects of the local topography and of the morphology of the animal may increase fertilization success above that predicted by this theoretical model.

The higher viscosities measured for female gametes than for male gametes in each species seems to be inconsistent with the fact that clumps of gametes spawned by females are dispersed more rapidly than those spawned by males in two of the three species, *E. mathaei* and *T. gratilla* (Table 3), and that clearance rates of gametes do not differ for males and females at low velocities (Figs 6 and 7). Indeed, at high velocities, clearance rates for female gametes are faster than for those of males (Figs 6 and 7) although female gametes are more viscous (Fig. 1). This observation suggests that semen may have a constituent that is not soluble in sea water. In addition, eggs of each of these species have a thick jelly coat (Harvey, 1956). It is possible that the high viscosities measured for the female gametes are the result of the measurement of the viscosity of this jelly coat in combination with the affinity of eggs for one another.

The shear-thinning of gametes

This study clearly demonstrates that gametes of these three sea urchin species exhibit shear-thinning behaviour. The highest viscosities and relative differences in the viscosities occur at the lowest shear rates (Fig. 1). At the highest shear rates, the viscosities of the gametes are relatively low and the differences among them become minimal. Shear-thinning may provide advantages in performance at both ends of the spectrum of shear rate. For example, the rapid changes in the viscosities of gametes occur at shear rates below $6\,\mathrm{s^{-1}}$. Shear velocities in excess of $0.08\,\mathrm{m\,s^{-1}}$ for females and $0.05\,\mathrm{m\,s^{-1}}$ for males are required to exceed these shear rates in the field. The habitats where *T. gratilla* and *E. mathaei* were collected had shear velocity ranges that are below this. Thus, the greatest changes and differences among the viscosities of gametes occur in the range of shear rates that the animals are most likely to experience in the field. However, for *C. atratus*, which inhabits intertidal habitats that seasonally experience large breaking waves, field shear velocities probably exceed these values at times (see Denny, 1988, for a discussion of water flow in intertidal habitats).

At the lower shear rates experienced in the field, the high viscosities of gametes may allow the formation of clumps or strings and may allow retention of gametes on the surfaces of the animals, thereby resulting in increased fertilization rates over those that could be expected if gametes were simply released in a plume, as discussed above. At high shear rates, low viscosities of gametes provide a performance advantage for sea urchins by decreasing the power required to maintain the gamete flow through the

gonoducts during spawning. Since the diameter of the gonopore also influences the power required to extrude gametes, there may be an interesting relationship between gamete viscosity and gonopore diameters in sea urchins.

Whether the high viscosities and differences in viscosity measured in this study are the result of selection in habitats that differ in wave action is not clear. *C. atratus* and *E. mathaei* are more closely related to one another than to *T. gratilla* (*E. mathaei* and *C. atratus* are in the family Echintometridae whereas *T. gratilla* is in the family Echinidae). Nonetheless, the gametes of *E. mathaei* and *T. gratilla*, species that share a common habitat, display greater similarities to one another in terms of viscosity than do the gametes of *E. mathaei* and *C. atratus*. This suggests that selection might play a role in influencing the viscosity of gametes in these species. It is also possible, however, that the viscosity of gametes is a plastic characteristic or that it is determined by some other factor, such as type of food available in a habitat.

Although, the shear-thinning observed for gametes of these sea urchins may well provide the performance advantages described above, shear-thinning is common in other biological fluids, including blood (Frey-Wysling, 1952; Leyton, 1975; Fung, 1981) and milk (Leyton, 1975). Therefore, either these fluids are all under similar selection pressures (i.e. have advantages in performance when viscosity is high at low shear and low at high shear) or they share some characteristics that result in shear-thinning, such as the suspension of particles in a liquid (see Fung, 1981, for a discussion of blood flow). The lack of a temperature effect on viscosity over the range 10–26 °C suggests either that the gametes have some property regulating viscosity or that the factor that controls the viscosity of the fluids is independent of temperature, such as the suspension of particles.

There is a wide variety of free-spawning marine invertebrates that occur in habitats varying a great deal in their exposure to waves. In addition, the physical characteristics of gametes and the materials in which they may be packaged vary greatly between species. Results from this study suggest that the physical properties of gametes may be under selection to decrease the rapid spread of gametes during free spawning. The results also indicate that there may be multiple mechanisms for egg and sperm contact in free-spawning species. To understand more about the role of these physical characteristics and mechanisms of sperm–egg contact, further research in this area is required and this may lead to an understanding of the evolution of gamete packaging in marine invertebrates.

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