Revised Estimates of the Effects of Turbulence on Fertilization in the Purple Sea Urchin, *Strongylocentrotus purpuratus*

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Turbulent water motion can either aid or hinder external fertilization in aquatic organisms. On one hand, turbulence provides the mixing necessary to bring eggs and sperm together; on the other, the forces imposed by turbulent eddies may interfere with the attachment of sperm to eggs and may even damage zygotes. Mead and Denny (1) explored this dichotomy by measuring the efficacy of fertilization in the purple sea urchin (Strongylocentrotus purpuratus) *while gametes were subjected to sheared flow in a Couette cell. When calculated rates of turbulent energy dissipation exceeded 100 W/m³, fertilization and early development were severely affected. Dissipation rates of this magnitude are common in breaking waves, and Mead and Denny therefore concluded that turbulent flow could be a substantial environmental hindrance to sexual reproduction in nearshore urchins. However, the rates of energy dissipation calculated by Mead and Denny for the Couette cell were erroneously small. Here we use direct measurements of energy dissipation rates to show that fertilization success can exceed 80% even when dissipation is as high as 2200 W/m³, higher than the dissipation likely to be found in breaking waves. Thus, many energetic flow environments that were previously thought to be detrimental to external fertilization may instead be benign or advantageous.*

The majority of benthic marine invertebrates reproduce sexually *via* external fertilization. The effectiveness of this strategy has been the subject of much recent research, and the roles of water motion in "fertilization ecology" have been debated (for a review, see (2)). Given the limited swimming capabilities of sperm, if adults are separated by more than a few centimeters some water motion is required to bring sperm and eggs together. To this end, turbulence (and the bulk mixing that it causes) are advantageous. However, this mixing can occur only if water is sheared, and as a result, turbulence inevitably imposes viscous forces on gametes (3). If these forces inhibit the attachment of sperm to eggs or damage the gametes or zygote, the advantages of mixing can be negated. Whether turbulence is an aid to fertilization or a hindrance thus depends in part on where the line is drawn between effective mixing and shear-induced damage.

Mead and Denny (1) and Mead (4) examined this issue by measuring the ability of sea urchin gametes to fertilize under the controlled imposition of turbulent flow. Eggs of *S. purpuratus* were introduced into a volume of water contained in the space between two coaxial cylinders (a Couette cell, Fig. 1A). When the outer cylinder was rotated, the water was sheared, and, by varying the rate of rotation, the shear stress imposed on gametes could be controlled. Once the apparatus was up to speed, sperm were introduced at a concentration sufficient to result in 80%–90% fertilization in still water, and fertilization was allowed to proceed for 2 min. A volume of KCl solution was then introduced into the cell to prohibit further fertilization, and the percentage of eggs fertilized was determined.

With these results in hand, the turbulence intensity in the Couette cell was compared to the intensity characteristic of the wave-swept habitat in which *S. purpuratus* is found. The translation from laboratory to field conditions was made *via* the turbulent dissipation rate ε (measured in W/m³), the rate at which turbulence-induced shear stress in the water converts the energy of the moving fluid into heat (1, 5):
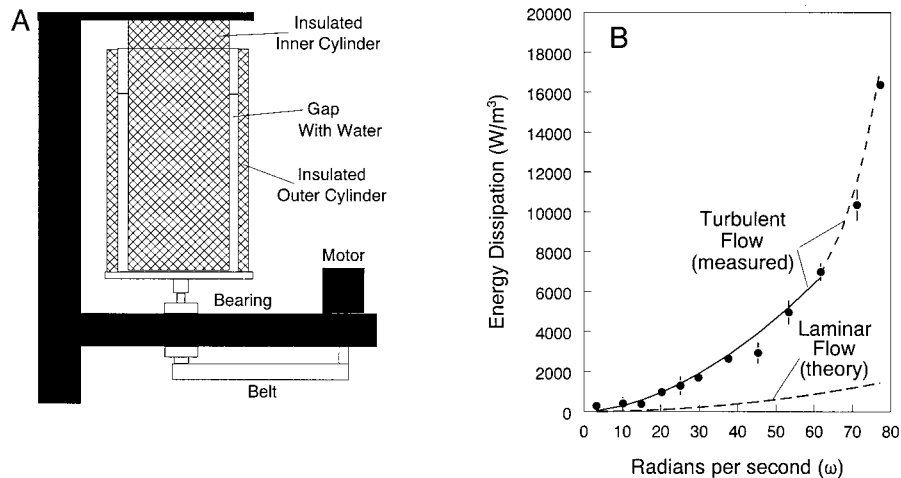
Figure 1. (A) The Couette cell of Mead and Denny (1) was modified to measure the rate at which water was heated by turbulent energy dissipation. The water bath was removed from the stainless steel inner cylinder, and a temperature-sensing device (Analog Devices AD590) and resistive heating element (Watlow Columbia K010030C5-0009B) were affixed separately to its inner surface. To minimize heat loss, the inner cylinder was then filled with insulating foam and the outer cylinder wrapped in a layer of closed-cell foam. Enough deionized water (100 ml) was added to the gap between the inner and outer cylinders to rise above the top of the heating element. The instrument was calibrated by passing a known current through the known resistance of the heating element (thereby determining the rate at which heat was injected into the system), and noting the rate at which temperature increased. An Agilent E3630A power supply was used to measure the current passing through the heating element, and an Agilent 34401 6-digit multimeter was used to monitor the voltage output from the temperature sensor and to interface with a computer. During calibration, the outer cylinder was rotated at a rate just sufficient to ensure that heat was well mixed throughout the water, but not so rapidly as to cause measurable heating *via* viscous dissipation. After an initial nonlinear temperature fluctuation associated with the thermal mass of the apparatus, temperature increased linearly at a rate proportional to the heat produced by the resistive element. In this fashion, the rate of temperature increase in the device could be interpreted in terms of the watts of heat energy injected for each cubic meter of water in the cell, in effect giving a measure of the specific heat capacity of the apparatus. The heating element was then turned off, and the apparatus was allowed to return to room temperature. Subsequently, the outer cylinder was rotated at a series of constant angular velocities; in each case the rate at which the water was heated was noted. By comparing these rates to the calibrated rates obtained with the heating element, we were able to measure total energy dissipation as a function of the angular velocity of the outer cylinder. The results shown here (B, filled circles ± 95% CL) indicate that energy dissipation in the cell is much larger than would be predicted by Eq. 2 if only dynamic viscosity, μ, were present. The solid line is a power curve fit to the data, excluding the two points at highest ω ($\varepsilon = 4.918\omega^{1.751 \pm 0.134 \text{ (SEM)}}$, $r^2 = 0.961$). The remaining two points are better fit with a 5th-order polynomial calculated using all of the data ($\varepsilon = -46.903 + 112.22\omega - 10.337\omega^2 + 0.494\omega^3 - 0.00875\omega^4 + 0.000056\omega^5$, $r^2 = 0.998$). The dashed line depicting dissipation in laminar flow is taken from Eq. 2 with ν set to zero and a small correction for the effect of shear beneath the end of the inner cylinder: $\varepsilon = 0.276\omega^2$.

$$\varepsilon = \frac{\tau^2}{\mu} \qquad (1)$$

Here τ is the shear stress (Pa) and μ is the dynamic viscosity of the water (1.24×10^{-3} N s m^{-2} at 12 °C, the temperature at which the experiments were carried out). Shear stress can be related in turn to the motion of the Couette cell,

$$\tau = A\frac{\omega r}{h} \qquad (2)$$

where ω is the angular velocity of the outer cylinder (in radians/s), r is the inner radius of the outer cylinder (5.4 cm in this case), and h is the radial separation between cylinders (3.5 mm). A is the total viscosity of the fluid (5). Mead and

Denny (1) assumed that A was equal to μ, which is true if flow is laminar. However, when flow is turbulent (as it was in the Couette cell),

$$A = \mu + \nu, \qquad (3)$$

where ν is the eddy viscosity (5). Eddy viscosity is typically much larger than μ (5). Therefore, by neglecting ν, Mead and Denny grossly underestimated the rate at which energy was dissipated during their experiments.

The magnitude of A is difficult to predict with any precision; theoretical estimates vary over a wide range (*e.g.,* 6, 7, 8). To obtain accurate values, we therefore measured energy dissipation rates directly by monitoring the rate at which water was heated in the Couette cell as a function of
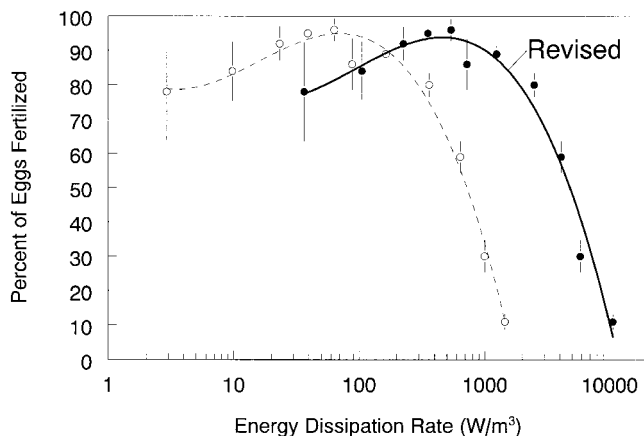
Figure 2. Fertilization results of Mead and Denny (1) and Mead (4) replotted using the new, empirical estimates of energy dissipation rate (filled circles). For comparison, the data are also plotted using the previous, erroneous dissipation estimates (open circles).

angular velocity (for the details of the measurements, see the caption to Fig. 1). These measurements were made in the same Couette cell used for the fertilization experiments (Fig. 1A).

Energy dissipation rates in the Couette cell fell within the range predicted by turbulence theory (6, 7, 8), and are indeed far in excess of those calculated by Mead and Denny (Fig. 1B). These empirical results can be used to re-interpret the previous fertilization data (Fig. 2). The percentage of eggs fertilized increases with increasing ε up to a rate of about 600 W/m^3. This dissipation rate is greatly in excess of rates measured in the surf zone for waves 1 m high breaking on a gently sloping beach (10–100 W/m^3) (9), and is comparable to the predicted dissipation rate for 1-m-high waves on the steeper slope of a typical rocky shore (1, 10). Indeed, the percentage of eggs fertilized remains above 80% until ε exceeds 2200 W/m^3, a dissipation rate greater than that predicted for the 2-m-high waves that are typical of surf conditions on rocky shores (1, 11). Thus, the high dissipation rates measured in the Couette cell paint a different scenario from that previously presented: the line separating the "good" from the "bad" effects of turbulence-induced shear stress is shifted to much higher turbulence intensities. Only if gametes are subjected to the shear stresses associated with very large breaking waves is fertilization in the purple sea urchin likely to be severely inhibited; under more typical conditions (waves < 2 m high), the mixing associated with turbulence may be advantageous in that it brings sperm and eggs into contact. This reinterpretation of laboratory results could help explain why some invertebrates (*e.g.,* gastropods) spawn preferentially when sea conditions are rough (12).

It is important to bear in mind that other free-spawning species may have different sensitivities to intense turbulence. Note, also, that this interpretation of "good" and "bad" ignores

at least two potentially detrimental effects of turbulent mixing. Although mixing is commonly required for contact between sperm and eggs, it also can result in the dilution of gametes (13, 14), and the lower the co-occurring concentration of gametes, the lower the probability of fertilization (15). Thus, unless gametes are confined in a manner that reduces dilution (in a surge channel or tide pool, for instance (7)), turbulence may still be disadvantageous for all but the lowest levels needed for effective mixing. Second, in the rare instances when sperm are present in high concentration, an increase in mixing due to turbulence could increase polyspermy, and thereby decrease the fraction of viable zygotes.

Acknowledgments

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