Reference: *Biol. Bull.* **227:** 33–39. (August 2014)

Relative Effects of Gamete Compatibility and Hydrodynamics on Fertilization in the Green Sea Urchin *Strongylocentrotus droebachiensis*

LOUISE T. KREGTING[1,2,*,†], FLORENCE I. M. THOMAS[2], ANNA L. BASS[1], AND PHILIP O. YUND[1,‡]

[1]*Marine Science Center, University of New England, Biddeford, Maine 04005; and* [2]*Hawai'i Institute of Marine Biology, University of Hawai'i, Kane'ohe, Hawai'i 96744*

Abstract. Intraspecific variation in gamete compatibility among male/female pairs causes variation in the concentration of sperm required to achieve equivalent fertilization levels. Gamete compatibility is therefore potentially an important factor controlling mating success. Many broadcast-spawning marine invertebrates, however, also live in a dynamic environment where hydrodynamic conditions can affect the concentration of sperm reaching eggs during spawning. Thus flow conditions may moderate the effects of gamete compatibility on fertilization. Using the green sea urchin *Strongylocentrotus droebachiensis* as a model system, we assessed the relative effects of gamete compatibility (the concentration of sperm required to fertilize 50% of the eggs in specific male/female pairs; F_{50}) and the root-mean-square of total velocity (*u*rms; 0.01–0.11 m s^{-1}) on fertilization in four locations near a spawning female (water column, wake eddy, substratum, and aboral surface) in both unidirectional and oscillatory flows. Percent fertilization decreased significantly with increasing *u*rms at all locations and both flow regimes. However, although gamete compatibility varied by almost 1.5 orders of magnitude, it was not a significant predictor of fertilization for most combinations of position and flow. The notable exception was a significant effect of gamete compatibility on fertilization on the aboral surface under unidirectional flow. Our results suggest that selection on variation in gamete compatibility may be strongest in eggs fertilized on the aboral surface of sea urchins and that hydrodynamic conditions may add environmental noise to selection outcomes.

Received 22 January 2014; accepted 23 June 2014.

* To whom correspondence should be addressed. E-mail: l.kregting@qub.ac.uk

† Current address: School of Planning, Architecture and Civil Engineering, Queens University of Belfast, Northern Ireland, BT22 1PF, UK.

‡ Current address: The Downeast Institute, P.O. Box 83, Beals, Maine 04611.

Abbreviations: *u*rms, root-mean-square of total velocity; F_{50}, concentration of sperm required to fertilize 50% of the eggs in specific male/female pairs; OWT, oscillatory water tunnel; UF, unidirectional flume.

Introduction

Incompatibility between gametes occurs both within and among species of broadcast-spawning invertebrates and is often mediated by variation in gamete recognition proteins (Palumbi and Metz, 1991; Palumbi, 1994). The selective forces operating on this variation are thought to include a mix of conspecific processes and reinforcement to avoid hybridization (Palumbi, 1994; Levitan and Ferrell, 2006; Evans and Sherman, 2013). Within a species, variation in compatibility among male/female pairs potentially results in differential success in fertilization because less compatible individuals can achieve equivalent fertilization levels only at higher sperm concentrations (McCartney and Lessios, 2002; Rawson *et al.,* 2003; Levitan and Ferrell, 2006; Slaughter *et al.,* 2008). Specific male/female pairs should thus exhibit different levels of fertilization when environmental conditions are held constant.

Many broadcast-spawning invertebrates, however, live in energetic coastal environments, where hydrodynamic conditions create spatial and temporal variation in the concentration of sperm reaching eggs. In general, fertilization is expected to decline in higher velocity and more-turbulent flows (Pennington, 1985; Levitan *et al.,* 1992; Yund and Meidel, 2003; Thomas *et al.,* 2013). In addition, small-scale turbulent processes may result in regions of high concentrations of sperm and eggs within the water column

(Crimaldi and Browning, 2004) surrounded by an overall background where gamete concentrations are diluted to biologically irrelevant levels (Levitan *et al.,* 1992; Levitan and Young, 1995; Wahle and Peckham, 1999). Gamete characteristics can result in the formation of packets of gametes that travel at high concentrations downstream of a spawning animal (Thomas, 1994; Meidel and Yund, 2001). The presence of the spawning animal also creates deviations in the water flow that can trap gametes at high concentrations in recirculating eddies or on the substrate around a spawning female (Meidel and Yund, 2001; Kregting *et al.,* 2013; Thomas *et al.,* 2013). Thus there can be considerable variation in gamete concentration within the water column and at different locations on and around a spawning female, even under similar flow conditions.

Most work to date on the consequences of variation in gamete compatibility to fertilization has focused on its implications for individual reproductive success and fitness, in either the context of hybridization (*e.g.,* Rawson *et al.,* 2003) or conspecific processes (*e.g.,* Levitan and Ferrel, 2006; Evans and Sherman, 2013). Less attention has been paid to how conspecific variation in compatibility interacts with environmental factors that also affect fertilization. While gamete compatibility may affect individual reproductive success, hydrodynamic conditions and gamete properties may moderate the expected impact. Here we compare the effects of hydrodynamics and gamete compatibility on fertilization in the green sea urchin *Strongylocentrotus droebachiensis* in unidirectional and oscillatory laboratory flows.

Unidirectional flow is typical of tidally controlled environments (in the sense that flow is generally unidirectional for the duration of a spawning event), whereas oscillatory motion is generated by wind and swells in coastal environments. Flow type has implications for fertilization processes in broadcast-spawning invertebrates owing to the frequency of disruptions of the shear layers at the surface of the organism. Shear layers are constant in unidirectional flow but are continuously disrupted in oscillatory flow, resulting in high shear stresses whose frequency is determined by the wave period. Further levels of turbulence associated with different types of flow control the rates of gamete diffusion. Thus flow type will influence processes such as gamete movement around the parent, the threshold when gametes are ablated away from the parent, and the level of turbulence in the fluid environment, all of which in turn influence fertilization (Kregting *et al.,* 2013; Thomas *et al.,* 2013).

Experimental evidence has shown that the viscous spawn of sea urchins is often retained on the surface of the parent (Yund and Meidel, 2003; Thomas *et al.,* 2013). As the gametes leave the parent, they can be retained in the wake of the animal or sink to the substrate where fertilization can take place. The importance of each location varies with velocity and flow type (oscillatory and unidirectional; Kregting *et al.,* 2013; Thomas *et al.,* 2013). Therefore, we assessed fertilization at different locations on and around a spawning female (water column, wake eddy, substratum, and aboral surface) over a range of flow conditions to ask whether gamete compatibility or hydrodynamics explained more of the variance in fertilization, and whether relative effects varied among locations.

Materials and Methods

Experimental design

Experiments were conducted in a unidirectional flow-through flume (hereafter referred to as unidirectional flume [UF]) and a fully enclosed acrylic oscillatory water tunnel (OWT) that provided two different discrete experimental hydrodynamic treatments (unidirectional and oscillatory; see Thomas *et al.,* 2013, and Kregting *et al.,* 2013, for detail). Within each type of flow, we ran a series of trials over a range of flow velocities (UF and OWT) and period (OWT). All experiments were conducted between 6 March and 1 May 2007 at the University of New England, Maine. The working section of the UF was a rectangular box, 3 $\times$ 0.5 m, filled to a water depth of 0.2 m; the OWT had a working section measuring 3.6 (L) $\times$ 0.31 (W and H) m. In both flow types, male and female sea urchins were secured to the bottom of the flume in the center of the working section. The male was placed 1 m upstream of the female, a distance that represents spacing in a relatively low-density spawning scenario (Levitan *et al.,* 1992) and permits comparison with other studies (Meidel and Yund, 2001; Kregting *et al.,* 2013; Thomas *et al.,* 2013).

Water velocity was measured with an Acoustic Doppler Velocimeter (SonTek ADVField) to characterize the flow at the height of the female urchin when the urchin was not present (0.04 m above the substrate). Velocity data were recorded for 2 min at a sampling frequency of 16 Hz. For full details of the technical specifications and post-processing of the ADV measurements, see Kregting *et al.* (2013) and Thomas *et al.* (2013). To compare hydrodynamic effects in the two flumes, we calculated the root-mean-square of the total velocity (*u*rms, where *u* represents the velocity in the longitudinal component (m s^{-1})). The *u*rms of the total velocity is a measure of water velocity that contains the current, the magnitude of any wave-driven flow, and the turbulence with instrumental noise removed. We chose to use total *u*rms because past results indicate it to be a better predictor of fertilization than current, wave-driven flow, or turbulence alone (Kregting *et al.,* 2013). In the UF, 13 fertilization trials were conducted at a range of *u*rms (0.018–0.11 m s^{-1}). In the OWT, 20 fertilization trials were conducted at a range of hydrodynamic conditions with both period (T = 4.5–12.1 s^{-1}) and *u*rms (0.016–0.10 m s^{-1}) manipulated independently (see Kregting *et al.,* 2013). This range of *u*rms was expected to create a variation in fertilization of an order of magnitude as reported by Kregting *et al.* (2013) and Thomas *et al.* (2013). The *u*rms values used in the experiments were representative of the range of flow velocities typically observed in sea urchin habitats of the Gulf of Maine (Yund *et al.,* unpubl. data).

Sea urchins were collected by hand from the northern coast of Maine and kept in flow-through seawater tanks at ambient temperatures (2–8 °C). For each trial, several sea urchins with an average test width of 0.066 ± 0.009 SE m were randomly selected from the tanks and injected with about 3.0 ml of 0.5 mol l^{-1} potassium chloride (KCl) to induce spawning. Although KCl injection may cause animals to extrude gametes more rapidly than they would in a natural spawn (Levitan *et al.,* 1992), the rate-limiting step for gamete dispersal in our experiments was the rate at which viscous gametes were advected from the aboral surface (a process occurring on the scale of hours), not the rate of extrusion *per se* (a process occurring on the scale of minutes; Thomas *et al.,* 2013). One male and one female that spawned contemporaneously from at least four gonopores were selected, and samples of eggs ($n = 2$) and of sperm ($n = 1$) were collected prior to the placement of the urchins in a flume. One egg aliquot was immediately assayed to determine the presence of (1) fertilization membranes, or (2) immature or abnormal eggs. If more than 5% of the eggs showed abnormalities or the eggs were contaminated with sperm, the experiment was terminated and no data collected. The remaining aliquots were used to quantify gamete compatibility.

Gamete compatibility was assessed at the level of the phenotype (Palumbi and Metz, 1991; McCartney and Lessios, 2002) to integrate across the effects of variation at the bindin locus (Levitan and Ferrell, 2006) and other potential contributing loci (*e.g.,* Biermann *et al.,* 2004). To assess phenotypic gamete compatibility, "dry sperm" and eggs rinsed in aged seawater were refrigerated at 4 °C for 90 min after collection. Gamete compatibility was calculated as the sperm concentration required to achieve 50% fertilization (F_{50}; McCartney and Lessios, 2002) and determined for each pair of sea urchins used in fertilization trials as follows: 200 μl of eggs was pipetted into 4 ml of sterilized seawater in 20-ml scintillation vials ($n = 6$ per pair). Sperm from the male was diluted by mixing 100 μl of "dry" sperm in 900 μl of sterilized seawater in a dram (3.7 ml) vial. Five additional 10-fold serial dilutions were then tested. First, 100 μl of each dilution was pipetted into each vial containing eggs (bringing the total volume to 4.3 ml) and gently swirled. After a 5-min incubation at 4 °C, 1 ml of formaldehyde was added to each scintillation vial to terminate development. A 100-μl sample of sperm from the 3rd serial sperm dilution was added to 100 μl of gluteraldehyde for later determination of sperm concentration *via* a hemocytometer. Two replicates of each sperm count were conducted and averaged to increase the accuracy of sperm concentration estimates. The percentage of eggs fertilized in each vial was calculated from a random subsample of 100 eggs. F_{50} values were calculated from the relationship between fertilization and sperm concentration using a logit transformation for fertilization (McCartney and Lessios, 2002; Rawson *et al.,* 2003). Because gamete compatibility had to be assessed after the flume trials were conducted, all trials were blind with respect to F_{50}.

Fertilization trials

Fertilization was assessed as a time-integrated measure (Yund and Meidel, 2003). However, since retention of gametes on the surface of the adult changes depending on velocity (Thomas *et al.,* 2013), sampling intervals and duration of the trials were inversely related to velocity. Samples were evenly spaced (3 to 5 time points) throughout the time period in which gametes were present on the spawning animals (UF: 65–120 min; OWT: 60 min). At each sample time, eggs were sampled in quick succession from four locations in the following order: the water column, wake eddy, substrate, and aboral surface. In the UF, the water column was sampled for 1 min, 0.5 m downstream of the female and 0.1 m above the substrate, with a plankton net (11.5-cm diameter and 35-μm mesh size). Due to the physical constraints of the OWT, 1 liter of water was siphoned from the water column, 0.7 m downstream of the female, 0.1 m above the substrate. The wake eddy was sampled with a 50-ml syringe attached to an extended tube to avoid disturbance of the flow field surrounding the female. Within 2 min of collection, eggs sampled from the water column and wake eddy were filtered through a 35-μm mesh sieve and gently rinsed with aged (24–48 h) seawater into a 20-ml vial containing 2.5 ml of 37% formaldehyde. For the substrate and aboral surface, within less than 1 min of collection, eggs were carefully pipetted into a 20-ml vial containing 5 ml of aged seawater and 1.5 ml of 37% formaldehyde. Although the time required to process these samples might have resulted in some additional fertilization in the containers, sperm concentrations would have been very low. In addition, all samples were treated the same, so the technique could not have generated differences among trials. Fertilization levels were calculated as the percentage of a random subsample of at least 100 eggs (or the total sample if <100 eggs were present) for each sample collected for each time point (3–5) at each location (water column, wake eddy, substrate, and aboral surface). Samples with <10 eggs total (<4% of all the samples) were excluded from calculations of the weighted mean for each location (see next section).

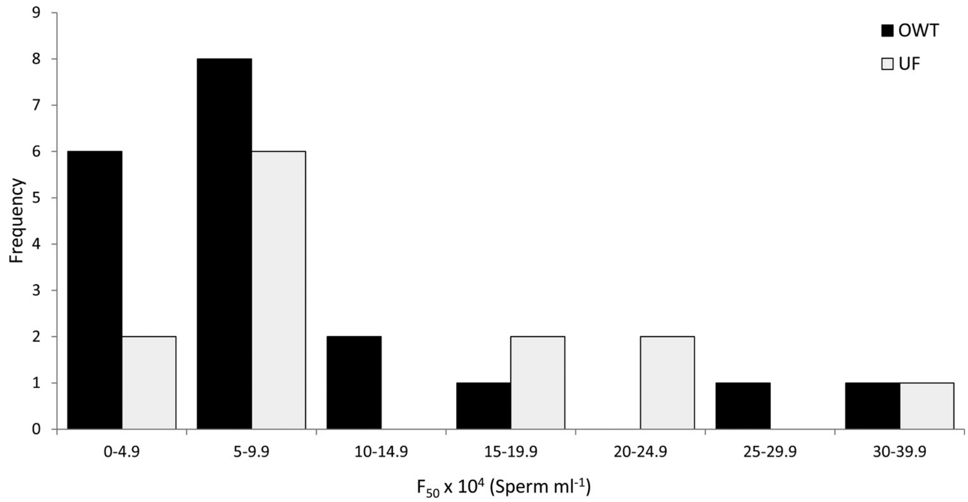

Figure 1. Frequency of gamete compatibility (F_{50}) for the pairs of male/female sea urchins. Black bars refer to the 19 experimental trials conducted in the fully enclosed oscillatory water tunnel (OWT), and white bars refer to the 13 experimental trials conducted in the unidirectional flume (UF). One outlier (>2 SD above the mean) was excluded from the OWT trials.

Effect of hydrodynamics and gamete compatibility on percentage of eggs fertilized

To assess how water motion (*u*rms) and gamete compatibility (F_{50}) influenced the percentage of eggs fertilized (PF) at each sampling location near the spawning female (water column, wake eddy, substrate, and aboral surface), we calculated a weighted mean whereby the PF at each time point was weighted by the sampling time. To determine the relationships of *u*rms and gamete compatibility (F_{50}) with the time-integrated PF at each location, enter method multiple regression analyses were performed. These analyses enter both the *u*rms and F_{50} values into the equation at the beginning of the analysis and must pass the tolerance criterion (0.001) in order to remain in the regression equation, where the tolerance is the proportion of the variance of a variable in the equation that is not accounted for by other independent variables in the equation. Period was not entered into the multiple regressions for oscillatory flows because period was shown to have no effect on fertilization in past experiments (Kregting *et al.,* 2013). Fertilization values were arcsine-transformed and *u*rms was natural log transformed to linearize the data. All other multiple regression assumptions were met. Analyses were performed using IBM SPSS 19.0 for Windows 2010, and alpha was set at $P < 0.05$.

Results

Gamete compatibility among individual pairs of sea urchins varied over almost 1.5 orders of magnitude (F_{50} range of 2.3–92 $\times 10^4$ sperm ml^{-1}) and exhibited a similar range of variation in both flow types (Fig. 1). One pair in the OWT trials exhibited a F_{50} value of 92 $\times 10^4$ sperm ml^{-1}, which was more than twice the magnitude of the next highest pair (39.7 $\times 10^4$ sperm ml^{-1}) and 2 SD above the mean for the group. Because this outlier had the potential to unduly affect the regression analyses, we analyzed the relationships with and without this point included. The results of the analysis did not change with exclusion, so it was excluded from the OWT analyses and from Figures 1 and 2 to provide greater clarity for the visual relationship between F_{50} and fertilization. Although there was a pronounced mode in the range of 3–10 $\times 10^4$ sperm ml^{-1}, the distribution of compatibility values was otherwise fairly flat (Fig. 1). Consequently, less compatible pairs required an order of magnitude or higher sperm concentration to achieve the same fertilization level as more compatible pairs.

In all locations, the level of fertilization was highly negatively dependent on *u*rms (Fig. 2, Tables 1 and 2). However, despite the considerable range of variation in phenotypic gamete compatibility (Fig. 1), the only significant effect of gamete compatibility on fertilization occurred on the aboral surface in unidirectional flow (Table 1). F_{50} was not a significant contributor to the multiple regression in any other location or in oscillatory flow (Tables 1 and 2). Because we conducted a number of similar regressions, we also applied a sequential Bonferroni correction (Rice, 1989) to adjust the table-wide alpha to 0.05 (Tables 1 and 2 combined). The effect of gamete compatibility on fertilization on the aboral surface in unidirectional flow was still significant ($P = 0.005$) after the Bonferroni correction (table-wide alpha = 0.05 when alpha for individual tests =

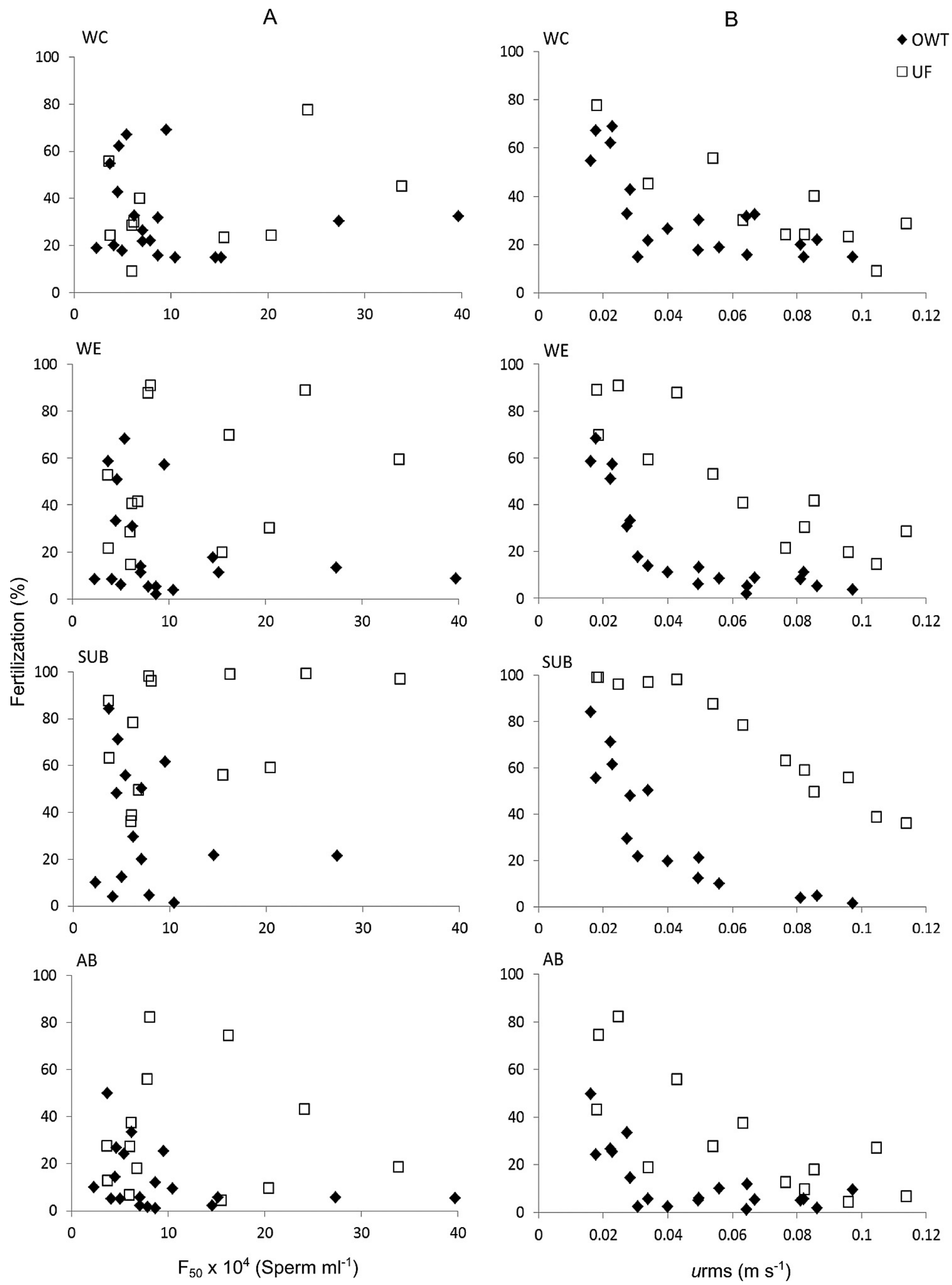

Figure 2. Mean percent fertilization as a function of gamete compatibility (F_{50}) (A) and *u*rms (m s^{-1}) (B) in the four sampling locations: water column (WC), wake eddy (WE), substrate (SUB), and aboral surface (AB) in the oscillatory water tunnel (OWT) and unidirectional flume (UF). Symbols represent weighted mean of all the time points (3–5).

0.006). As would be expected when trials were conducted blindly with respect to one variable (F_{50}), the predictor variables *u*rms and F_{50} were not correlated (Pearson product-momentum correlation $r^2 = -0.46$, df = 13, $P = 0.117$, and $r^2 = 0.11$, df = 20, $P = 0.647$ for the UT and OWT, respectively). Hence any potential effect of F_{50} was not subsumed by a relationship with *u*rms.

Discussion

The level of fertilization decreased with increasing water velocity in all locations, a result consistent with numerous earlier sea urchin studies (Pennington, 1985; Levitan *et al.,* 1992; Yund and Meidel, 2003; Kregting *et al.,* 2013; Thomas *et al.,* 2013). The lack of an effect of gamete

Table 1

Results of multiple regression analyses in unidirectional flow

	Unidirectional flow-through flume (UF)				Model			
Location	Variable	β	t	Sig.	Adj. R^2	df	F	Sig.
WC					0.72	9	12.31	< 0.01
	ln *u*rms	−1.02	−4.45	< 0.01				
	F_{50}	−0.25	−1.10	0.31				
WE					0.74	12	18.34	< 0.01
	ln *u*rms	−0.95	−5.79	< 0.01				
	F_{50}	−0.18	−1.0	0.31				
SUB					0.85	12	35.73	< 0.01
	ln *u*rms	−0.94	−7.52	0.01				
	F_{50}	0.001	0.01	0.99				
AB					0.80	12	25.55	< 0.01
	ln *u*rms	−1.03	−7.14	< 0.01				
	F_{50}	−0.52	−3.62	< 0.01				

The percentage of eggs fertilized in different locations—water column (WC), wake eddy (WE), substratum (SUB), and aboral surface (AB)—for the green sea urchin *Strongylocentrotus droebachiensis* was regressed on the predictor variables ln *u*rms and $F_{50.}$ Results are reported for the standardized coefficients (β), t value with significance for each predictor and the overall model (adjusted R^2, degrees of freedom, and F statistic).

compatibility (except on the aboral surface in unidirectional flow) is somewhat surprising. Earlier studies reported about an order of magnitude variation in fertilization under the range of flow conditions explored here (Kregting *et al.,* 2013; Thomas *et al.,* 2013), and the range of variation in sperm concentration required for 50% fertilization spanned more than an order of magnitude (Fig. 1). So the absence of a strong gamete compatibility effect in this study does not appear to be an artifact of comparing two effects of fundamentally different magnitudes.

Effects of gamete compatibility may have been limited to the aboral surface in unidirectional flow because, under these flow conditions, large egg piles form here over a range of flow velocities and last for a relatively long time (Yund and Meidel, 2003; Thomas *et al.,* 2013). The stationary nature of the egg piles might increase the probability that sperm encounter eggs and increase the opportunity for eggs to choose among sperm. In oscillatory flow, the increased disruptions of the shear layers close to the surface of the animal, owing to the magnitude and period of the oscillatory wave, lead to the rapid erosion of the egg piles so that eggs remain on the aboral surface for only a fraction of the time observed in unidirectional flows (Kregting *et al.,* 2013). Gamete compatibility depends in part on sperm and egg contact rates (Zimmer and Riffell, 2011), and the physical conditions on the aboral surface are likely to increase the chances of contact when flow is unidirectional.

Interestingly, fertilization on the aboral surface has not received much attention; the emphasis has mainly been on fertilization processes occurring in the water column. Thomas *et al.* (2013) showed that the aboral surface can contribute a large portion of successful fertilizations under a range of flow conditions. The results here further suggest that the aboral surface is likely the location where gamete compatibility plays more of a role than in other locations. Our result notwithstanding, one other study has detected effects of the bindin locus on water column fertilization in a close congener (Levitan and Ferrell, 2006). Therefore, to fully understand the role of gamete compatibility in fertilization, studies should be done under realistic flows and consider the role of locations other than the water column (*i.e.,* the aboral surface, substrate, and wake eddy) in overall fertilization success.

It appears likely that differences in gamete compatibility will have more obvious effects on fertilization outcomes when tested in urchins that experience comparable flow regimes and gamete mixing. Real-world flows are likely to reduce the magnitude of selection in any round of mating by adding a level of environmental noise to the outcomes. This result does not fundamentally change the importance of selection on compatibility, but it does indicate that local environmental conditions may play a major role in mediating the fitness consequences of compatibility.

Table 2

Results of multiple regression analyses in oscillatory flow

	Oscillatory water tunnel (OWT)				Model			
Location	Variable	β	t	Sig.	Adj. R^2	df	F	Sig.
WC					0.56	18	12.54	< 0.01
	ln *u*rms	−0.81	−4.94	< 0.01				
	F_{50}	0.12	0.71	0.49				
WE					0.79	18	35.28	< 0.01
	ln *u*rms	−0.91	−8.10	< 0.01				
	F_{50}	0.03	0.26	0.80				
SUB					0.84	14	38.64	< 0.01
	ln *u*rms	−0.92	−8.60	< 0.01				
	F_{50}	−0.04	−0.39	0.70				
AB					0.47	18	8.973	< 0.01
	ln *u*rms	−0.69	−3.83	< 0.01				
	F_{50}	−0.10	−0.57	0.58				

The percentage of eggs fertilized in different locations—water column (WC), wake eddy (WE), substratum (SUB), and aboral surface (AB)—for the green sea urchin *Strongylocentrotus droebachiensis* was regressed on the predictor variables ln μrms and $F_{50.}$ Results are reported for the standardized coefficients (β), t value with significance for each predictor and the overall model (adjusted R^2, degrees of freedom, and F statistic).

Acknowledgments

We thank E. Zimmermann, S. Stickley, and J. Zima for assistance in the laboratory. Funding was provided by the

National Science Foundation (OCE-04-25088 and OCE-13-33755 to P.O.Y. and OCE-04-24978 to F.I.M.T). This is contribution number 1590 from the Hawai'i Institute of Marine Biology and 9196 from SOEST.

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