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# EFFECTS OF FERTILIZATION DISTANCE ON MALE GAIN CURVES IN A FREE-SPAWNING MARINE INVERTEBRATE: A COMBINED EMPIRICAL AND THEORETICAL APPROACH

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Male gain curves describe the relationship between allocation to sperm production and male reproductive success and are central to models of sex allocation in hermaphrodites. Sperm competition is expected to result in more linear gains and select for increased allocation. We hypothesized that high sperm production in passively mating systems may also be the result of selection to enhance the ability to fertilize distant ova. Consequently, we explored the effect of distance on male gain curves in a free-spawning colonial ascidian. The performance of focal males that varied in sperm production was assayed at three distances via microsatellite markers. An advection-diffusion model was used to estimate sperm concentration gradients, to predict male reproductive gain integrated across multiple downstream females, and explore effects of hydrodynamic conditions. As distance increased, male reproductive success decreased and empirical gain curves became increasingly linear. Our model predicted that the expected net gain curve is relatively insensitive to variation in flow regime and will saturate much more slowly than if only a single, nearby distance is considered. Thus, high levels of sperm production may enhance fitness both in competitive situations and with increasing fertilization distance, highlighting the need to consider distance effects when evaluating gain curves.

**KEY WORDS:** *Botryllus schlosseri*, diffusion/advection model, fertilization distance, paternity, sex allocation.

Hermaphroditic plants and animals can potentially maximize reproductive success by varying the relative production of male and female gametes (Charnov et al. 1976). The shape of the function that describes the relationship between gamete allocation and resulting gender-specific reproductive success (the fitness gain curve) is an important component of sex allocation models (Charnov 1979, 1982; Campbell 2000; Scharer 2009). These relationships can be linear, decelerating (saturating), or accelerating,

and the shapes of the curves under varied ecological conditions dictate the selective pressures imposed on gamete allocation patterns (Charnov 1982; Lloyd 1984). The realization that ecological factors may influence how resources are allocated to male and female gamete production has made an important contribution to the theory of sex allocation (Charnov 1979, 1982; Lloyd 1984).

Female gain curves are generally expected to be linear (Brunet 1992). However, female gain curves may be nonlinear in plants and animals that experience sibling competition, resulting from poorly dispersed offspring (Maynard Smith 1978) or if the number of offspring a female can brood is physiologically limited

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by the amount of brood space available (Heath 1979; Strathmann et al. 1984). By contrast, male gain curves are expected to vary with diverse conditions, including individual size (Petersen and Fischer 1996), the size of the local mating group (Charnov 1982; Raimondi and Martin 1991; Lorenzi et al. 2006), the rate of self-fertilization (Charlesworth and Charlesworth 1981), pollinator behavior (Harder and Thomson 1989), and the intensity of sperm competition (Fischer 1981; Lloyd 1984; Petersen 1991). In particular, male gain curves are expected to be linear when sperm compete for access to ova (either postcopulation or externally), but to saturate in the absence of competitors (Charnov 1982) because the production of additional male gametes results in diminishing fertilization gains per sperm. For brooding, free-spawning marine invertebrates (animals that release sperm into the surrounding seawater), a linear gain curve has been demonstrated in a colonial bryozoan under experimental conditions in which intense sperm competition would have been expected (McCartney 1997). Similarly, the predicted variation in gain curves has been demonstrated in a colonial ascidian under a range of competitive conditions (Yund 1998).

However, an alternative hypothesis can be advanced for the fitness benefits of high levels of sperm production in passive mating systems, which in addition to the egg brooding, free-spawning invertebrates cited above (spermcasters sensu Bishop and Pemberton 2006) include sedentary broadcast-spawning marine organisms (organisms that release both egg and sperm) and wind-pollinated plants (Bishop and Pemberton 1997). Previous experiments have tested the reproductive gain of variable sperm (or pollen) production only for single, nearby females (Schoen and Stewart 1986; McCartney 1997; Yund 1998). But in natural populations, a male's reproductive success will be achieved via fertilization of ova brooded by females at varying distances from the male. Consequently, high levels of sperm production could enhance male reproductive success by increasing the probability that a male will fertilize distant eggs, even in the absence of nearby sperm competitors. The role that variation in sperm production plays in determining male reproductive success with distance has not yet been addressed. Male gain curves may saturate at nearby distances, but become linear with increasing distance, suggesting that distant matings will favor increased allocation to sperm production. Alternatively, male gain curves may saturate at all distances, suggesting that increased allocation to sperm production will not be favored by distant matings.

This article explores the effect of fertilization distance on male gain curves in the hermaphroditic colonial ascidian *Botryllus schlosseri*. We present empirical results from in situ fertilization studies in experimental populations in which the reproductive success of focal males that varied in sperm production was assayed at three distances, with microsatellite markers used for paternity assignment. We then used a simple advection-diffusion model to

predict the structure of the sperm plume in two dimensions (with diffusion and hence sperm dilution implicitly extending into the un-represented third dimension). Those model results were in turn used to predict the net fitness gain of males integrated across downstream females (based on a random distribution of females; hereafter termed the expected net gain curve) and to evaluate the effects of hydrodynamic conditions on expected net gain curves.

## Methods

### STUDY ORGANISM AND CULTURE

*Botryllus schlosseri* is a suspension-feeding marine invertebrate that can be found on hard substrates in shallow waters (including harbors) throughout much of the world (Van Name 1945). Colonies are composed of asexually produced zooids arranged in clusters or systems, with all zooids in a system sharing a common exhalant siphon (Milkman 1967). Colonies are cyclical hermaphrodites, and sexual reproduction occurs when sperm released from a male-phase colony fertilize eggs of a female-phase colony. Male and female phases alternate on a 7–12 days sexual cycle (temperature dependent; Grosberg 1982) linked to an asexual zooid replacement cycle. Eggs are fertilized at the beginning of a cycle and brooded internally until released as tadpole larvae at the end of a cycle. Sperm are freely spawned into the water column, beginning approximately 16 h after the start of a new cycle and continuing for several days, so colonies are functionally male throughout most of the sexual cycle (Stewart-Savage and Yund 1997). Because male and female phases are offset in time and colonies are unable to store sperm (Stewart-Savage et al. 2001), colonies are not able to self-fertilize (Stewart-Savage et al. 2001; Phillippi 2005).

Fertilization in *B. schlosseri* is extremely efficient, with dilute, long-lived sperm (Johnson and Yund 2004) captured throughout a prolonged period of egg viability (Stewart-Savage et al. 2001; Yund et al. 2007). Consequently, more than 85% of eggs are generally fertilized in natural populations (Phillippi et al. 2004). Although eggs are usually fertilized by sperm from males in their immediate vicinities (Yund and McCartney 1994; Yund 1995, 1998), the frequency distribution of dispersal distances for fertilizing sperm can be highly leptokurtic (Grosberg 1991) and eggs of isolated colonies can be fertilized up to 207 m from a population (Yund et al. 2007). Hence, it is virtually impossible to eliminate sperm competition in natural environments; sperm are present throughout the coastal habitats inhabited by this species.

*Botryllus schlosseri* colonies exhibit substantial variation in sperm production. At the level of the individual zooid, colonies vary in testis size, and there is a strong genetic component to this variation (Yund et al. 1997). Variation in testis size is highly correlated with actual sperm counts (Yund et al. 1997). Broad-sense heritability estimates for testis size range from 0.42 to 0.75

(Yund et al. 1997), and the narrow-sense heritability estimate for sperm production from a nested half-sib design is 0.28 (P.O. Yund, unpubl. data). Second, because each zooid in a colony produces sperm, total sperm production is also greatly affected by colony size, which varies through ontogeny. However, unlike most colonial invertebrates, *B. schlosseri* colonies do not grow indeterminately. Instead, colonies eventually reach a terminal size (generally associated with the onset of sexual reproduction), at which the initially rapid growth of the colony halts, and this size is then maintained over subsequent, asexual zooid replacement cycles (Grosberg 1988; Boyd et al. 1986). Colonies also exhibit variation in terminal size among genotypes (Yund et al. 1997), and when variation in sperm production per zooid and terminal size are integrated, total sperm production among terminal sized colonies varies by  $\sim 1$  order of magnitude (Yund 1998). Colonies also vary in allocation to egg production, with a three-way trade-off at the phenotypic level between male and female allocation and asexual growth (Yund et al. 1997).

#### EXPERIMENTAL MATING ARRAYS

Colonies for experiments were collected from two sites in the Damariscotta River, Maine (DM and CI; see Yund and Stires 2002 for location specifics), in May and June of 2005 and 2006. Colonies were explanted on glass microscope slides and initially housed in the flowing seawater laboratory at the University of Maine's Darling Marine Center before transfer to the flowing seawater laboratory at the University of New England's Marine Science Center. The colonies were maintained in flowing seawater and fed a mix of algae (*Isocrysis* sp. and *Dunaliella* sp.) until deployed in the field as experimental males or females. The colonies used in experiments were selected at stages in their reproductive cycles so that only the colony assigned the male role could fertilize the females. Females were selected to be in reproductive synchrony, so that none of the female colonies would have started to release sperm before the eggs of the other females were fertilized.

The cross-sectional area (length  $\times$  width) of 16 testes (one pair per each of eight zooids) of each experimental male was measured with an ocular micrometer. To ensure that this sub-sample included variation within testes pairs and between neighboring zooids (Yund et al. 1997), testes were measured in groups of four (located in four different systems throughout the colony), with each group consisting of the two pairs of testes in neighboring zooids. Testis cross-sectional area varied greatly among experimental male colonies, with colony means ranging from 0.054 mm<sup>2</sup> to 0.168 mm<sup>2</sup>. A relative index of total sperm production was then calculated for each colony as the mean size of the 16 measured testes multiplied by two times the number of zooids in the colony. Sperm production (or testis area) did not vary systematically between years ( $t = 1.23$ ,  $P = 0.24$ , d.f. = 13) or among deployment

dates within either year (2005:  $r = 0.52$ ,  $P = 0.14$ ; 2006:  $r = 0.17$ ,  $P = 0.74$ ).

Laboratory-cultured colonies were assembled onto experimental mating platforms constructed of acrylic plates (2.44 m  $\times$  0.30 m). Four replicate mating platforms were deployed at  $\sim 9$  m maximum depth in Saco Bay, Maine. The platforms were positioned along a 100-m transect and were separated by at least 20 m cross-current to restrict sperm contamination among platforms (although not all platforms were simultaneously occupied by colonies). The first platform on the transect was located 40 m cross-current from the only source of natural colonies in the area. This location did not completely isolate our experimental colonies from natural sperm sources (which is impossible to achieve in this species), but was designed to minimize background sperm. Experimental conditions were thus comparable to the competitor-free treatment of Yund 1998, in that local sperm competitors were absent, although more distant sperm sources were not eliminated. Under these conditions, local males are known to dominate nearby fertilizations (Yund and McCartney 1994; Yund 1998), but were expected to incur dilution effects with more distant females, with exogenous natural sperm contributing the balance of the fertilizations. We thus tested distance effects on male gain curves within the context of a low-competition background. The real-world analogy of our experimental design is a low-density assemblage of colonies on the periphery of a larger population. Because the distances over which we measure the gain curves were orders of magnitude shorter than the distance separating our platforms from each other and the nearest population, we assume that the background concentration of sperm did not vary among distance classes.

Each acrylic plate was mounted 4 cm above concrete patio blocks (3 blocks/plate) and oriented with the long-axis of the plate parallel to bidirectional tidal flow. A focal male (experimental male of measured sperm production) was mounted 0.16 m from one end of the platform, and three females were mounted, respectively, at 0.1, 0.6, and 1.6 m from the male. Females were thus positioned so that they received sperm from the focal male for half of each tidal cycle, and our analysis and subsequent model focus entirely on this half of the cycle. During the other half of the cycle, sperm from the focal males would have been advected away from the experimental platforms and toward uninhabited areas. Each trial generated a fertilization success estimate for a single focal male at each distance, and sperm production varied among trials. By testing three fertilization distances simultaneously, this design controls for potential individual male effects in the comparison among distance classes, although individual male effects may still contribute to the variation within a distance class. Colonies were placed in the field  $\sim 48$  h prior to the anticipated time of sperm release and onset of egg viability. The number of eggs brooded by each female colony was counted

prior to field deployment and controlled within a limited range (mean =  $55.2 \pm 21.7$  SD;  $n = 45$ ). Each trial was deployed in the field for 6–8 days, then collected and returned to the laboratory. An incision was made in each zooid of each female colony, the unfertilized eggs and developing embryos were counted, and 32 embryos were surgically extracted for paternity analysis. Fifteen replicate trials were deployed in the field during August and September of 2005 and July–September of 2006.

#### PATERNITY ASSIGNMENT

Paternity assignment was performed with three microsatellite loci that varied in levels of polymorphism from 3 to 30 alleles, but combined provide circa 95% accuracy in assignment (Johnson and Yund 2007). Genomic DNA was extracted from fresh adult tissues using the PureGene kit (Qiagen, Valencia, CA) (Marine Invertebrate Extraction protocol #00690). DNA was extracted from frozen embryos by incubating samples in ddH<sub>2</sub>O and 20  $\mu$ g of Proteinase K (New England BioLabs, Ipswich, MA) for 2 h at 55°C, followed by incubation at 90°C for 8 min. Each sample was genotyped at three loci using the touch-down PCR protocol detailed in Johnson and Yund (2007). PCR products were electrophoresed on a LiCor 4200 Global IR<sup>2</sup> DNA Analyzer (LI-COR, Lincoln, NE), scored using the SAGA 2 software (LI-COR), and confirmed visually.

Progeny genotypes were compared with known maternal and focal male genotypes. All offspring carried at least one maternal allele. Neither this study nor our previous paternity work with microsatellites (Johnson and Yund 2007, 2008) has produced evidence of null alleles at these loci. Offspring that carried a match to a focal allele at each of the three loci were assumed to have been fathered by the focal male. Offspring with genotypes that were inconsistent with the focal male were assumed to have been fertilized by exogenous sperm from natural colonies. Contamination from nonfocal males with identical genotypes was possible, but should have been equal at all distances and hence should not have confounded the overall results.

#### STATISTICAL ANALYSIS

The success of the focal male colonies in fertilizing eggs brooded by each experimental female colony was calculated as the percentage of sampled embryos fertilized by the focal male and expressed as percent fertilization. Basing our analyses on the actual number of eggs fertilized would have only contributed additional variation from differences in egg production among females. To evaluate the shape of the male gain curve at each distance, we fitted power functions ( $y = ax^b$ ; Charnov 1979, 1982) to the data. The exponent of the power function ( $b$ ) describes the shape of the curve. If that exponent is significantly  $<1$  or  $>1$ , reproductive success is either a saturating or accelerating function of sperm production (respectively). If the exponent does not differ significantly

from 1, reproductive success is judged to be a linear function of sperm production. For each distance we performed a one-tailed Student's  $t$ -test to evaluate whether the exponent differed significantly from a value of one. The data within each distance class are truly independent and meet all regression assumptions, because each fertilization value is associated with a unique male, even though the same males are represented in different distance classes. However, any comparison of exponents among distance classes is compromised by this lack of independence among distance classes. Nevertheless, the outcome of the preceding  $t$ -tests led us to an unplanned comparison among exponents, subject to the noted independence limitations. The exponent for the 1.6-m distance class was compared to the exponents for the 0.1-m and 0.6-m distance classes by one-tailed Student's  $t$ -tests.

#### MODELING SPERM PLUMES

A simple two-dimensional advection-diffusion model (Denny 1988) that has been employed in numerous other fertilization studies (e.g., Levitan et al. 1991; Levitan and Young 1995; Metaxas et al. 2002) was used to model sperm plumes. Terrestrial pollen plumes have traditionally been modeled by somewhat different equations (e.g., Jackson and Lyford 1999) because the analog to pollen sedimentation does not occur in marine systems. In essence, for a given rate of sperm release, this model predicts the resulting spatial gradients of sperm concentration for a given mean water velocity and three-dimensional mixing scenario. Mean velocity provides the advection function, whereas a measure of turbulent fluctuation (friction velocity) and dispersion coefficients provide the basis for modeling diffusion in three dimensions. Sperm are assumed to be transported as passive particles because sperm swimming speed is trivial compared to water movement across a very broad range of environmental conditions (Denny 1988). From equation 10.34 of Denny (1988), the concentration of sperm ( $c$ ) at any point  $\times$  distance downstream of the male and  $y$  distance off-axis was predicted to be

$$c(x,y) = \left( \frac{Q\bar{u}}{2\pi\alpha_y\alpha_z u_*^2 x^2} \right) \cdot \exp - \left( \frac{y^2 \bar{u}^2}{2\alpha_y^2 u_*^2 x^2} \right)$$

where

$Q$  = rate of sperm release (sperm  $s^{-1}$ )

$\bar{u}$  = mean flow velocity ( $m s^{-1}$ )

$\alpha_y$  = dispersion coefficient on the  $y$ -axis (perpendicular to flow)

$\alpha_z$  = dispersion coefficient on the  $z$ -axis (vertical)

$u_*$  = friction velocity ( $m s^{-1}$ )

In turn,  $u_*$  was estimated from the mean flow velocity and bottom roughness via the relationship (Jumars and Nowell 1984):

$$u_* = \frac{\bar{u}k}{\ln\left(\frac{z}{z_0}\right)}$$

where

$k$  = von Kármán's constant = 0.4

$z$  = height above the substrate (m)

$z_0$  = substrate roughness length (m).

Dispersion coefficients were held constant at  $\alpha_y = 2.2$  and  $\alpha_z = 1.25$  (per Denny 1988), height  $z = 0.08$  m (reflecting the height of the experimental colonies above the substrate), and sperm release  $Q = 9$  sperm  $s^{-1}$  (based on average per-zooid sperm release values at the peak of male phase from Stewart-Savage and Yund 1997, and an approximate colony size of 100 zooids). To reflect the range of flow conditions commonly encountered by *B. schlosseri* colonies in nature, we varied  $z_0$  between 0.01 and 0.001 m and varied  $\bar{u}$  between 0.02 and 0.15  $m s^{-1}$ . These parameter values represent a combination of empirical data collected adjacent to natural populations (e.g., Yund and McCartney 1994) and generally accepted values for similar habitats (e.g., Kushnir 2005). Although this model only predicts sperm concentrations on the  $x$ - $y$  plane, it is important to note that the inclusion of the vertical ( $z$ -axis) dispersion coefficient ( $\alpha_z$ ) allows sperm to leave that plane and enter the third dimension (i.e., sperm are not artificially "trapped" on the plane that is explicitly modeled). This model can be extended to predict sperm concentrations in all three dimensions, but sperm moving further up the  $z$ -axis are biologically irrelevant for the fertilization of benthic organisms inhabiting the  $x$ - $y$  plane at  $z = 0.08$  m.

#### PREDICTION OF EXPECTED NET GAIN CURVES

Output from the sperm plume model was used to predict the expected net gain curve for focal males under different flow conditions, on the assumption that female-phase colonies are randomly distributed within the plume. We first used our model, parameterized to match the physical conditions of our field site ( $\bar{u} = 0.02$   $m s^{-1}$  and  $z_0 = 0.001$  m) and to predict sperm concentrations at 0.6, 1.0, and 1.6 m from the focal males. Next, we assumed that the empirical gain curves measured at each of those three distances would describe the relationship between sperm production and reproductive success at any point on the  $x$ - $y$  plane where the predicted plume had a similar sperm concentration. Because the predicted sperm concentrations varied among empirical sample locations by at least an order of magnitude, the tolerance for similarity could be set at half an order of magnitude without leading to overlap in the three corresponding sperm concentration classes. The three empirical gain curves were thus applied to the portion of the  $x$ - $y$  plane predicted to receive from 0.2 to 5 times the sperm at each empirical sample location. The relative area of the portion of the  $x$ - $y$  plane corresponding to each empirical gain curve was then calculated, and those values were used to weight the contribution of each distance-specific gain curve to the expected net gain. We ignored any contribution from portions of the plume with lower sperm concentrations than the empirical sample locations. Male

reproductive success was quite low at our 1.6-m station (see results), so the excluded areas farther down- and cross-stream are unlikely to contribute substantially to total reproductive success, in spite of the relatively large areas involved.

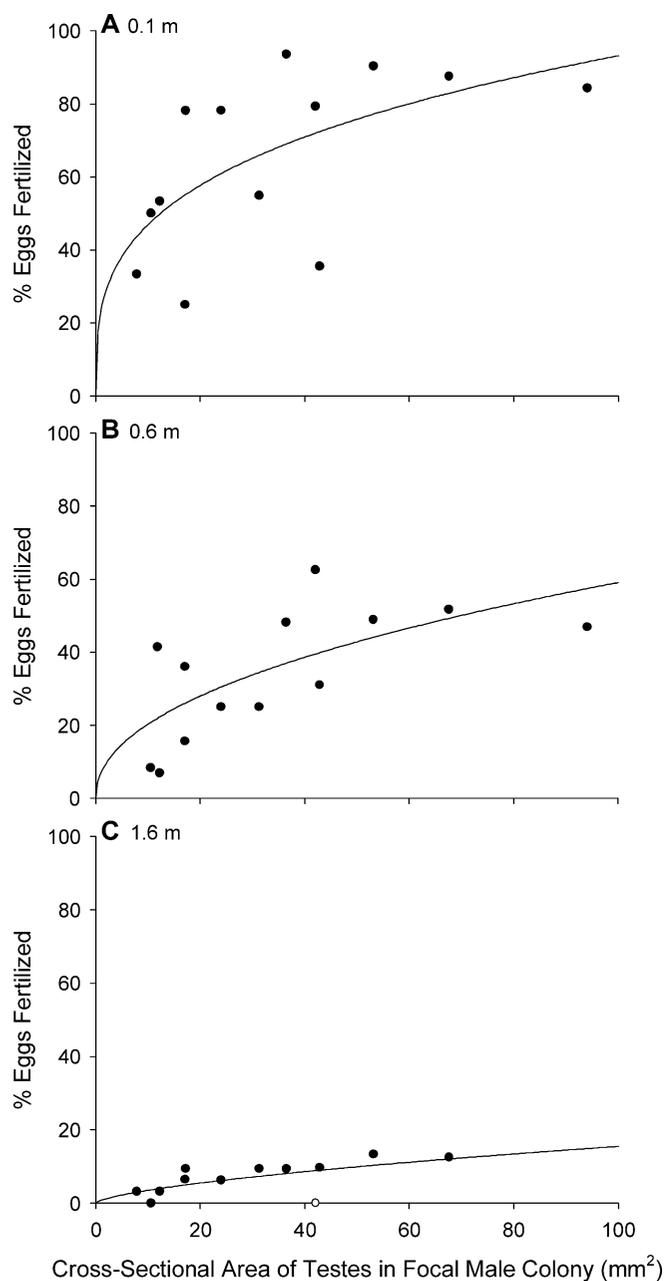
To assess the effects of variation in hydrodynamic conditions on expected net gain, we applied the same approach under other model parameter ( $\bar{u}$ ,  $u_*$ , and  $z_0$ ) combinations. Empirical gain curves were assumed to apply to different regions of sperm concentration as established in the initial parameterization that matched our field conditions, but the area of the sperm plume for each predicted concentration was calculated from the model results under different parameter combinations. Because variation in the model parameters changes the shape of the predicted sperm concentration gradients, the actual area associated with each empirical gain curve varied with model parameters. The relative portion of the plume associated with each gain curve was then used to weight the contribution of each empirical gain curve to the expected net gain. Results are reported only for the extreme combinations of parameter values, but were qualitatively similar for all parameter combinations explored within the stated ranges.

## Results

### EMPIRICAL GAIN CURVES

Of the 45 female colonies in the 15 field trials of this experiment, we were not able to collect data on the progeny of seven due to the death of the females during deployment (two females at 0.1, two females at 0.6, and three females at 1.6 m distance). For the remaining 37 female colonies, we were able to assay total fertilization (from all sperm sources; calculated on the basis of the number of eggs brooded prior to deployment and the number of embryos brooded upon return to the laboratory, minus any unfertilized eggs brooded) for 30 colonies. Accurate predeployment egg counts could not be obtained for eight colonies because of minor debris between the colony and the glass slide. In this subset of 30 colonies, overall fertilization was very high at all three distances (0.1 m distance, mean = 96.6%  $\pm$  0.13 SE; 0.6 m distance, mean = 95.9%  $\pm$  0.14 SE; 1.6 m distance, mean = 97.6%  $\pm$  0.15 SE) and did not differ significantly among distances ( $F$  ratio = 0.37;  $P = 0.69$ ). Because of the very high fertilization levels, the absence of variation among distance classes, and our inability to accurately measure total fertilization in the other eight colonies, all paternity analyses are expressed as the percentage of the fertilized eggs fathered by each focal male, rather than the percentage of the initially available eggs fertilized (sensu Yund 1998). Given the high fertilization levels, these two estimates will generally agree within 5%.

At all three distances there was a significant, positive relationship between total sperm production and fertilization success of the focal male (Fig. 1; Table 1). At the nearest distance (0.1 m), male fertilization success started to level off above a moderate



**Figure 1.** The relationship between focal male testis production and male fertilization success for females positioned at three different distances (A, 0.1; B, 0.6; C, 1.6 m). Distance between the male and female increases from top to bottom. A possible outlier in panel C is identified by the open symbol.

sperm production level (Fig. 1A), with the exponent of the power function significantly  $<1$  (0.30; Table 1), indicating a nonlinear, strongly saturating relationship. At the intermediate distance (0.6 m), male fertilization success started to level off at a slightly lower sperm production (Fig. 1B), with an exponent in the power function still significantly  $<1$  (0.47; Table 1), again indicating a nonlinear relationship. Finally, at the farthest distance (1.6 m), male fertilization success was overall extremely low (Fig. 1C).

**Table 1.** Power function regression ( $y=ax^b$ ) of male fertilization success ( $y$ ) on testis production ( $x$ ) for three distances.

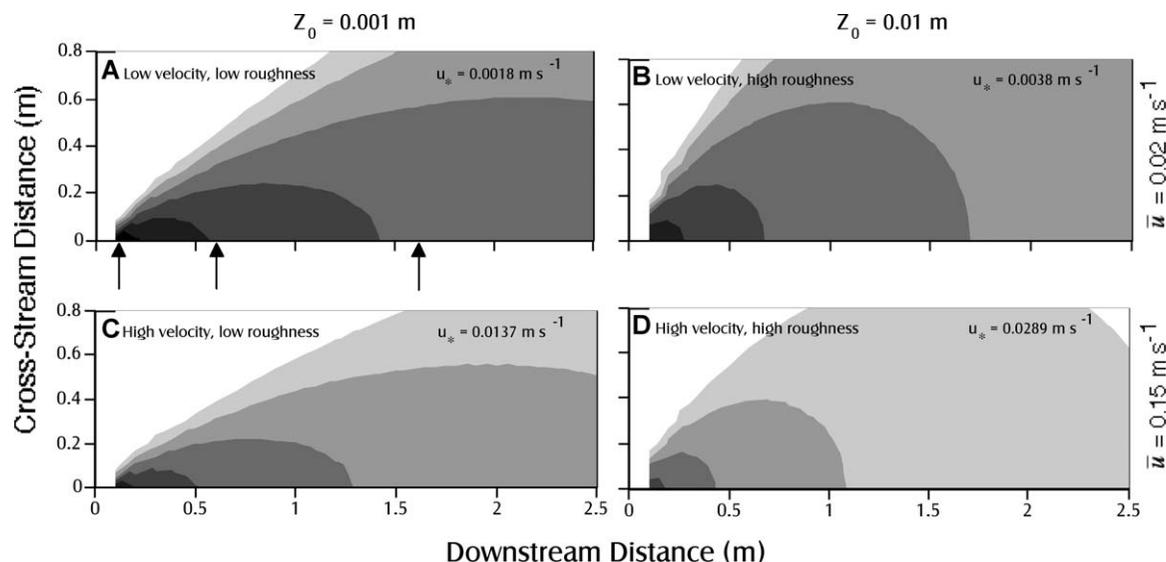
Statistic	0.1 m	0.6 m	1.6 m
Coefficient of determination ( $r^2$ )	0.38	0.47	0.54
$F$ ratio ( $P$ )	7.86*	10.11**	6.90*
Coefficient ( $a \pm SE$ )	$23.68 \pm 10.51$	$6.97 \pm 4.34$	$0.94 \pm 0.55$
Exponent ( $b \pm SE$ )	$0.30 \pm 0.12$	$0.47 \pm 0.16$	$0.65 \pm 0.15$
$t$ ( $p$ )	5.81****	3.25***	2.28*

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ .

The exponent of the power function was marginally  $<1$  (0.65; Table 1), again indicating a nonlinear saturating relationship. One extreme datum could have been considered an outlier (Fig. 1C, open symbol), but this exclusion would have had negligible effects on the exponent and significance level. Although the male gain curves were technically saturating at all three distances in this study, the relationship clearly became progressively more linear with increasing distance (exponent increases from 0.30 to 0.65; Table 1). The exponent for the 1.6 m distance was significantly higher than the exponent for the 0.1 m distance ( $t = 1.84$ , 23 d.f.,  $P = 0.039$ ), but did not differ significantly from the 0.6 m exponent ( $t = 0.82$ , 23 d.f.,  $P = 0.21$ ).

#### SPERM PLUMES AND CONSEQUENCES FOR EXPECTED NET GAIN CURVES

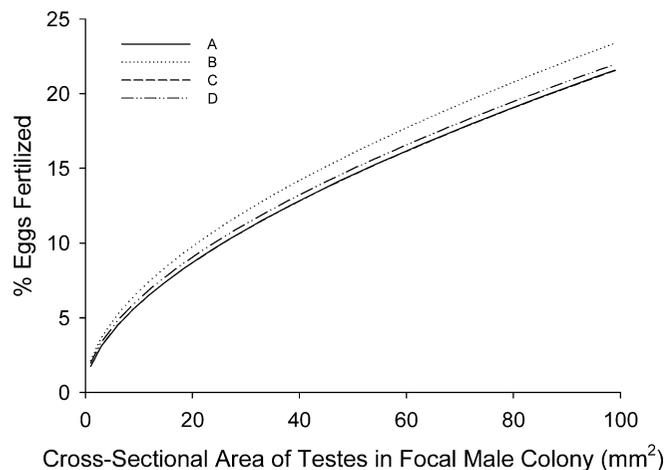
As previously recognized, variation in mean flow velocity ( $\bar{u}$ ), friction velocity ( $u_*$ ), and roughness length ( $z_0$ ) substantially altered concentration gradients within our predicted sperm plumes (Fig. 2). An increasing mean velocity reduced sperm concentration throughout the plume (Fig. 2, panels C and D compared to A and B), while increasing roughness length and friction velocity produced a broader plume with sperm concentration decreasing more rapidly with distance (Fig. 2, panels B and D compared to A and C). However, this substantial variation in plume shape and concentration gradient had a negligible effect on our estimates of expected net gain curves (Fig. 3). Under the parameter conditions corresponding to our empirical data, we predict that a male's reproductive gain will be nonlinear, but saturate only slightly at high sperm production (Fig. 3). The expected net gain is more linear than the gain at closer distances (Fig. 1, panels A and B) because as the sperm plume expands, a large area (and hence more females) contribute reproductive gains that are more linear. Moreover, this result appears relatively insensitive to variation in hydrodynamic conditions (Fig. 3). Even though the shape of the plume changes dramatically with hydrodynamic conditions (Fig. 2), the gain curve approaches linearity at relatively short distances, so the linear relationship dominates spatially, no matter what the plume shape.



**Figure 2.** Predicted two-dimensional distributions of sperm concentration under four sets of hydrodynamic conditions. In all panels, darker shading represents increased sperm concentration on a logarithmic scale, with each gradation in shading representing an order of magnitude change in sperm concentration (ranging from  $10^4$  to  $10^0$  sperm  $\text{mL}^{-1}$ ). The arrows in panel A indicate the three locations at which empirical data were collected. The substrate roughness length is denoted  $z_0$ , friction velocity is represented by  $u_*$ , and  $\bar{u}$  represents the mean velocity.

## Discussion

We have followed past workers in fitting power functions to our male gain curve data. Power functions offer a convenient mechanism for testing linearity (by providing an exponent that can be tested against a value of 1) and allow us both to interpret our work within the existing framework of sex allocation theory (e.g., Charnov 1979, 1982) and compare it to past empirical work (e.g.,



**Figure 3.** Composite, expected net male gain curves, assuming that females are randomly distributed within the sperm plume. Four lines correspond to the hydrodynamic conditions in the four panels of Figure 2, but the line corresponding to the conditions in Figure 2C is largely obscured by the line corresponding to the conditions in Figure 2A. The coefficients of these power functions vary between 0.53 and 0.58.

Schoen and Stewart 1986; McCartney 1997; Yund 1998). However, there is no biological basis for the choice of power functions over other possible saturating functions (such as sigmoidal or logarithmic functions)—power functions simply have convenient mathematic properties (Scharer 2009). Furthermore, we have no statistical basis for selecting one saturating function over another. For example, log functions fit to both our current and previously published data (Yund 1998) yield virtually identical coefficients of determination and an equally random distribution of residuals (unpublished analyses). Nevertheless, the choice of saturating function does have important ramifications when extrapolating beyond the data range. At higher sperm production levels, power functions converge on 100% fertilization, so the implicit assumption is that males could theoretically produce enough sperm to absolutely monopolize fertilizations at any distance (or level of sperm competition, or other ecological condition). By contrast, log functions asymptote at some maximum fertilization level  $<100\%$ , which is consistent with an assumption that males cannot fertilize all eggs, at least under some range of conditions. Unfortunately, we have not been able to test between these important alternatives because the levels of sperm production required to do so do not exist in nature (at least, not in our study organism). Although the choice of saturating functions remains an intriguing problem for future resolution, the remainder of our discussion is conducted within the conventional framework of using power functions to represent gain curves.

To the best of our knowledge, this is the first study to systematically examine the role that variation in sperm production

plays in determining male reproductive success with distance against a competitive background in a passive mating system. Male fertilization success decreased with distance at all sperm production levels, and although male gain curves at all sampled distances were statistically nonlinear, the relationship between sperm production and reproductive success became progressively more linear with increasing distance (Fig. 1). A comparison with male gain curves under variable levels of local sperm competition (Yund 1998) reveals some important similarities. First, increasing sperm production resulted in an increase in focal male reproductive success at all distances treatments (Fig. 1) and competitor treatments. Second, the gain curve for the nearest distance assayed in this study and the noncompetitor treatment (assayed at 5 cm; Yund 1998) are similar, although quantified by different techniques (allozyme vs. microsatellite paternity markers) and in two different sites (the Damariscotta River estuary vs. Saco Bay) located ~100 km apart, suggesting some generality to the pattern. Finally, the male gain curves became progressively more linear with both increasing distance (Fig. 1) and increased sperm competition. These results suggest that increased allocation to sperm production will convey a fitness advantage both for mating over increasing distances and under conditions of increased sperm competition. This comparison does not appear to be compounded by differences in the background sperm availability between studies. As with our current study, the sperm competition manipulation was conducted in the presence of some non-local sperm, and fertilization levels attributable to contaminating sperm were comparable. Only the local level of sperm competition was manipulated, and even the nominally competitor-free treatment was exposed to a background level of nonlocal sperm (Yund 1998).

Understanding whether sperm competition or fertilization distance is the more prevalent selective pressure in nature is a logical next step, but sperm competition and fertilization distance are two very different ecological factors that are not mutually exclusive and each likely to fluctuate in space and time, making it difficult to disentangle their relative importance. In spite of the isolation of our mating arrays from natural populations, both sperm dilution and sperm competition may have contributed to the decline in male fertilization success with distance (Fig. 1); eggs brooded by distant females were largely fertilized by sperm from exogenous males. However, exogenous males would have been orders of magnitude more distant from the females than the focal males, so our experimental design reduced sperm competition to the extent feasible in this model system. An ideal experiment might have manipulated fertilization distance and level of sperm competition simultaneously, but the resulting gain curve under increased sperm competition would have been virtually impossible to measure at our farthest distance. It is likely that conditions that intensify one selective pressure decrease the other, and vice

versa. Sperm competition is expected to have larger effects at high male or population densities (Yund and McCartney 1994), where male proximity effects are likely to minimize distant matings (Yund 1998). However, in less competitive situations (e.g., on the periphery of a population or within a low-density population) long mating distances are likely to contribute more to total male reproductive success (Yund et al. 2007).

When integrated throughout the area of the sperm plume, the expected net male reproductive gain we estimated was not linear, but saturated less steeply than when only a specific, shorter distance was considered (Fig. 3 vs. Fig. 1A). The predicted insensitivity to flow conditions (Fig. 3) may seem surprising, given how dramatically concentration gradients within the sperm plume change with flow conditions (Fig. 2). However, our estimates of the expected net gain are based on the relative ratio of areas within the plume that match the sperm concentrations under which our empirical data were collected. Even though the absolute areas of specific sperm concentrations change greatly with flow conditions (Fig. 2), the relative areas of the three relevant regions do not. If our assumption that sperm concentration dictates the shape of the gain curve is valid, then the predicted insensitivity to flow conditions is probably robust. Because the expected net male reproductive gain will integrate across some limited area of steeply saturating gain and much larger areas of more linear gain, a net reproductive gain that is mildly saturating appears to be an inevitable consequence of increasing gain curve linearity with distance.

The influence of fertilization distance on the evolution of simultaneous hermaphroditism in marine invertebrates and terrestrial plants has not previously been explicitly considered, although at least two plant allocation studies assume distance effects (Burd and Allen 1988; Eppley and Pannell 2001). The mildly saturating expected net gain that we predict as a consequence of integrating across multiple distances poses a minor challenge for sex allocation theory. Hermaphroditism is thought to have evolved by maximizing total individual reproductive success under conditions of diminishing fitness gains via male function (Charnov 1979, 1982). Under conditions of a saturating male gain curve, individuals are selected to shunt additional resources into reproduction as a female, and combined sexes are favored (Charnov et al. 1976; Charnov 1982). Although the mildly saturating expected net male gain curve that we report here is technically consistent with this hypothesis, the degree of saturation appears far less than generally assumed by models or suggested by previous, distance-specific empirical data (compare the degree of saturation in Fig. 3 with empirical data in Schoen and Stewart 1986; Yund and McCartney 1994; 1997; Yund 1998, and model assumptions in Charnov 1979, 1982). This pattern suggests that the fitness benefits to be obtained by shunting resources to female reproduction are perhaps more minor than traditionally expected.

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