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MALE REPRODUCTIVE SUCCESS IN SESSILE INVERTEBRATES: COMPETITION FOR FERTILIZATIONS[1]

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Abstract. Recent in situ fertilization studies of free spawning and brooding marine organisms have focused almost exclusively on the yield of fertilized ova (female reproductive success). As a consequence, we know little about the factors that determine male reproductive success. If marine organisms compete for fertilizations (as do many terrestrial organisms), then a male's reproductive success should be reduced by the presence of other males. We tested this hypothesis via in situ experiments employing allozyme markers for both a colonial ascidian (*Botryllus schlosseri*) and a bryozoan (*Celleporella hyalina*). Under moderate density conditions, the presence of closer male-functioning colonies reduced the fertilization success of more distant males in both species. In *C. hyalina,* male fertilization success also increased with allocation to sperm production. In addition, selfing rates in this species were negatively correlated with the abundance of outcross sperm. These results suggest that male reproductive success in sessile marine invertebrates must be assessed as a function of the gamete output and spatial distribution of other males in a population, and that the performance of isolated males may yield overestimates of male fertilization success in natural populations.

Key words: ascidian; bryozoan; fertilization ecology; paternity; self-fertilization; sex allocation; sperm competition.

INTRODUCTION

Sexual reproduction via the release of gametes into the water column is widespread among marine invertebrates (Giese and Kanatani 1987) and fishes (Breder and Rosen 1966). Despite the prevalence of this mode of reproduction, relatively little is known about fertilization under natural conditions. Such information is critical to a variety of ecological and evolutionary problems. For example, larval supply to benthic marine populations may be limited by processes affecting either larval survivorship or fertilization, and the relative importance of these alternatives cannot be evaluated until reliable estimates of egg fertilization rates are available (Pennington 1985, Denny and Shibata 1989). Similarly, estimates of individual fitness in marine species have typically not considered processes impacting fertilization success (Levitan 1991). In addition, for sessile and sedentary taxa, lack of information on fertilization has hindered the study of spatial mating patterns and the effects of such patterns on levels of inbreeding, degrees of kinship, and spatial scales of gene flow (all areas of active research in analogous terrestrial plant systems).

Recent in situ studies of fertilization in both sessile and mobile marine organisms have focused almost exclusively on determinations of the yield of fertilized ova (but see Grosberg 1991), hereafter termed female fertilization success. Consequently, factors such as distance from a sperm source (Pennington 1985, Yund 1990, Levitan 1991, Babcock and Mundy 1992, Brazeau and Lasker 1992), flow regime (Pennington 1985, Yund 1990, Petersen et al. 1992), population density (Levitan 1991, Levitan et al. 1992), gamete age (Oliver and Babcock 1992), and microhabitat (Petersen et al. 1992) are known to affect female fertilization success under field conditions. These findings have provided valuable insight into some of the factors that impact the efficiency of fertilization in the marine environment.

Absent from these studies, however, is comparable information on the individual contribution of different males to the fertilization of broods or spawns of eggs, hereafter termed male fertilization success. As a consequence, little is known about the ecological determinants of paternal success in marine organisms. In addition, the effect of variation in male fertilization success on the evolution of reproductive characters and alternative modes of reproduction has not yet been explored. An analogous state of affairs characterized terrestrial plant reproductive ecology in the 1970s (Bertin 1988). More recent considerations of both female and male contributions to fitness are thought to have revitalized the study of floral evolution (Wilson et al. 1994).

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The single recent study that examined male fertilization success in a marine invertebrate utilized allozyme markers to assay variation in the success of single males as a function of distance within a dense population (Grosberg 1991). This approach provides an accurate assessment of an individual male's fertilization success in a dense field population. However, the absence of a male density manipulation and a means to assign paternity to competing males prevents an explicit consideration of the impact of other males in the vicinity. Synchronous spawning is common among sessile marine invertebrates (Campbell 1974, Giese and Kanatani 1987), possibly as a consequence of strong selection on the timing of gamete release to enhance female fertilization success (Denny 1988). Thus, multiple males may release sperm that have the potential to fertilize an egg, thereby creating opportunities for males to compete for fertilizations. Although sperm competition has long been anticipated among fishes that spawn in swarms (Warner and Robertson 1978), and recent authors have speculated upon its occurrence in marine invertebrates (Ghiselin 1987, Strathmann 1990), empirical evidence in any marine system is lacking. In contrast, sperm competition appears to be common in most major groups of terrestrial organisms. In terrestrial plant systems, for example, pollen competition has been shown to be widespread (Mulcahy 1974, 1983, Snow 1986) and has been suggested to have a major impact on the evolution of mating systems (Mulcahy 1979).

Sperm competition in marine invertebrates will occur if sperm released by one male reduce the number of fertilizations obtained by another male. In marine systems, sperm competition may occur as a form of exploitation competition and does not require direct interaction among sperm. One individual's utilization of a resource (unfertilized eggs) may remove some portion of that resource from the pool available to another individual. If paternity is assayed purely on the basis of the percentage of embryos fathered by a given male, sperm competition may appear to be an inevitable consequence of sperm release by more than one male. Each male is likely to fertilize some portion of the resulting embryos, leaving the remaining male(s) with $<100\%$ of the fertilizations (termed "fair raffle competition" in copulating animals; Parker et al. 1990). However, fertilizations acquired by one male need not be obtained at the expense of other males. All evidence to date indicates that egg fertilization rates in marine invertebrates are typically $\ll 100\%$ under a wide range of field conditions (Pennington 1985, Yund 1990, Levitan 1991, Babcock and Mundy 1992, Levitan et al. 1992). As long as all available eggs are not fertilized by one male, then sperm from an additional male may simply result in more eggs being fertilized. While the first male now fathers $<100\%$ of the embryos that are produced, he may have fertilized the same number of eggs. In this scenario, no competition for fertilizations has occurred. Sperm are the limiting resource, not unfertilized eggs.

In this paper we explore aspects of sperm competition in two very different species of sessile, colonial invertebrates: *Botryllus schlosseri,* a colonial ascidian, and *Celleporella hyalina,* a cheilostome bryozoan. We present evidence from in situ fertilization experiments employing allozyme markers that indicates that the presence of a male-functioning colony near a female-functioning colony can reduce the fertilization success of a more distant male-functioning colony. The similarity of the competitive effects in these two very distantly related species suggests that competition for fertilizations may be a broadly occurring phenomenon in brooding invertebrates. We also demonstrate (in *C. hyalina*) that fertilization success in a competitive setting can vary as a function of male reproductive effort, and that selfing rates may be inversely proportional to the amount of outcross sperm available.

Materials and Methods

Overview

All work was conducted at the facilities of the University of Maine's Darling Marine Center in Walpole, Maine, during the summer and fall of 1992. All experiments utilized allozyme markers to determine the paternity of brooded embryos in trials in which different males had the opportunity to fertilize eggs. Due to differences in the reproductive biology of the two study species, we employed different methods and experimental designs for each. Consequently, we present our methods for the two species separately. To justify some aspects of the design of our mating experiments, we also analyzed the flow regime at our study site.

Botryllus schlosseri

B. schlosseri is a colonial stolidobranch ascidian that is common in the shallow subtidal zone throughout New England. This species inhabits a wide variety of firm substrata, including algae, rocks, bivalve shells, and man-made structures (Gosner 1971). Colonies are hermaphroditic with sequential female and male phases occurring in repetitive sexual cycles that are linked to an asexual zooid replacement cycle. Eggs are fertilized as a new asexual generation of zooids replaces an old generation (Milkman 1967), and once fertilized, develop into embryos that are released from the maternal colony at the end of the cycle (Grosberg 1991). Eggs that are not fertilized degenerate (P. O. Yund and M. A. McCartney, *personal observation*). Sperm production commences $\approx$2–3 d after eggs are fertilized (Milkman 1967). Self-fertilization is thus prevented by the temporal separation of male and female gamete production and the synchronization of the sexual cycle within a colony (Milkman 1967). The sexual–asexual cycle takes 7 d to complete in southern New England (Milkman 1967, Grosberg 1988) and 8–9 d in the colder

water of the Gulf of Maine (P. O. Yund and M. A. McCartney, *personal observation*).

In all experiments we assayed paternity on the basis of electromorphs at the GPI (glucose-6-phosphate isomerase) locus. Electrophoresis was performed using cellulose acetate membranes (Titan III, Helena Labs, Beaumont, Texas) run horizontally for 30 min at 250 V and 20°C. Gels were run under a 0.0025 mol/L citrate–0.025 mol/L phosphate, pH 6.4 buffer system and stained by a method modified from Richardson et al. (1986). All tissue samples were homogenized in grinding buffer (Grosberg 1987) prior to loading. Previous breeding experiments with *B. schlosseri* have demonstrated that electromorphs at the GPI locus segregate as Mendelian alleles (Sabbadin 1982). We named alleles by their relative mobility on the gel under standard conditions. The most common allele was designated GPI-100, and other alleles were designated with a value representing percentage mobility relative to that of the most common allele.

We obtained colonies for mating experiments by screening a natural population adjacent to our experimental site (40 m distant) for the presence of colonies that were homozygous for GPI alleles. Data from this screening procedure were also used to assess allele frequencies in the natural population. Prior to screening, colonies were collected from the field as adults, established in culture on glass microscope slides, and housed in a flowing seawater system. Colonies grow rapidly under these conditions without supplemental food sources. After colonies attained a minimum size (10–20 zooids), a portion of each colony (3–4 zooids) was excised for screening. The remaining tissue from homozygous colonies was returned to the flowing seawater system to permit further growth prior to field experimentation. Colonies were repeatedly subdivided as they grew to yield multiple copies of the same genotype.

Mating arrays were assembled with colonies that possessed various combinations of electrophoretic markers. We selected colonies that were entering either male or female phase (according to the criteria of Milkman 1967) for use in our experiments. The designation of gender employed throughout the remainder of this paper thus reflects each colony's functional gender at the time of an experiment. We employed three treatments consisting of a control (female only; $n = 8$; Fig. 1C), a competitor-absent treatment (one female colony flanked by a male colony in a distant position on both the upstream and downstream side; Fig. 1B, D), and a competitor-present treatment (one female flanked by two males, one each in distant and nearer positions, on both the upstream and downstream sides; Fig. 1A, E). In all cases, male colonies in symmetrical upstream and downstream positions of the female were two copies of the same genotype. These experimental treatments were repeated with two different spacing distances, which were selected (on the basis of earlier work; Grosberg 1991) to generate different degrees of overlap in the likely fertilization ability of the two males in the high-density treatment. In the close-spacing experiment, nearer males were positioned 5 cm from the female and distant males were positioned 10 cm from the female ($n = 7$ males in each treatment; Fig. 1D, E). In the expanded-spacing experiment, nearer males were positioned 10 cm from the female and distant males were positioned 20 cm from the female ($n = 6$ males in each treatment; Fig. 1A, B).

Mating arrays were housed in the field on rectangular acrylic plates (15 × 91 cm). Colonies growing on microscope slides were mounted on the plates with nylon bolts threaded into holes tapped in the acrylic. Colony size was controlled within each experiment (variation in average size between treatments was <9 % of the grand mean), but differed between spacing experiments due to variation in the size of stock laboratory colonies available when the two experiments were conducted (average male colony size: 63 zooids in the expanded-spacing experiment, 157 zooids in the close-spacing experiment). Experimental colonies were placed in the field 24–48 h prior to the onset of egg viability and sperm release and remained in the field for 6–7 d. Since eggs cannot be fertilized until the new generation of zooids in which they are contained commences feeding (Milkman 1967), experimental females could not have been fertilized prior to placement in the field. Male phase colonies release sperm over several days (Milkman 1967) and were selected at a consistent reproductive phase so that all males should have been releasing sperm when eggs first became viable to be fertilized. Replicates were placed in the field over a 6-wk period, but paired competitor-present and competitor-absent replicates were conducted simultaneously.

In the field, mating arrays were mounted 4 cm above cinder blocks (39 × 14 × 19 cm; L × W × H) that were partially filled with cement and oriented with their long axis parallel to current flow. These blocks were deployed in the field at a site in the Damariscotta River between Carlisle Island and the mainland, 1.8 km downriver from the marine laboratory. This site has a smooth, uniform bottom consisting of small rock cobble embedded in fine sediment, and *B. schlosseri* does not occur naturally at this location. However, it does occur nearby (nearest natural population is ≈40 m cross current) and can thrive at this site if provided with appropriate substrata. Thus the experiments were performed in a habitat suitable for growth and development, but where contamination from exogenous sperm was minimized. All blocks were spaced a minimum of 10 m apart, with spacing distances determined by deploying blocks on the intersection points of a rectangular rope grid (3 blocks × 5 blocks, or 20 m × 40 m). During the field experiments, the area around and between blocks was kept free of contaminating colonies by divers who patrolled every 2–3 d and re-

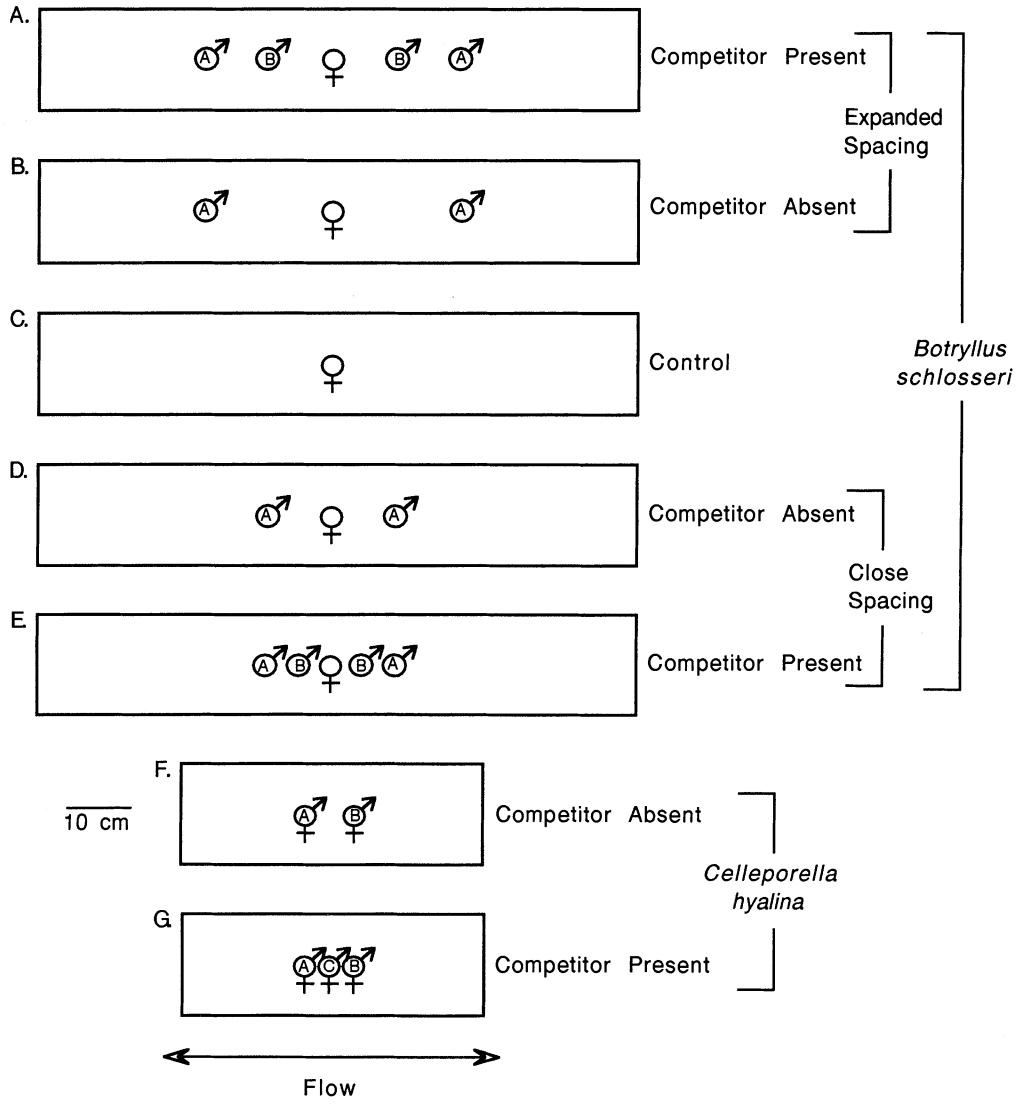

FIG. 1. Mating array diagrams illustrating experimental treatments for *Botryllus schlosseri* (A–E) and *Celleporella hyalina* (F, G) colonies. In all experiments with *B. schlosseri,* male genotypes were replicated in symmetrical positions on either side of the female. Three treatments were employed. Control plates (C) had only a lone female present to assay for background fertilization levels. Competitor-absent treatments had a female in the center with two copies of the same male genotype positioned 10 cm (D) or 20 cm (B) up and downstream. Competitor-present treatments had a female in the center with males at 5 and 10 cm (E) or 10 and 20 cm (A) up and downstream. A comparison within each pair of competitor-absent and competitor-present treatments (A and B; D and E) permits an evaluation of the fertilization success of a distant male in the presence and absence of a nearer male colony at each spacing interval. Only two treatments were employed in the experiment with *C. hyalina.* In the competitor-absent treatment, two colonies were separated by 5 cm (F), while in the competitor-present treatment, colonies were in these same positions but had a third colony inserted between them (G). As each *C. hyalina* colony is simultaneously male and female, these treatments permit an evaluation of the ability of each end colony to fertilize the other end colony in the presence and absence of a middle colony.

moved recruiting *B. schlosseri* colonies. Although juvenile *B. schlosseri* colonies were sometimes present in the area, often on the mating array blocks and plates themselves, these colonies were eliminated long before they reached sexual maturity and hence would not have served as alternative sperm sources.

When experimental colonies were returned to the laboratory from field experiments, 22–33 brooded embryos were dissected from each female colony for paternity analysis. We sampled embryos rather than later developmental stages to minimize any effects of post-fertilization selection on allele frequencies. Embryos were separately homogenized in 3 μL of grinding buffer contained in the wells of a 72-well micro-titer plate (Nunc, Naperville, Illinois), transferred to loading plates, and then applied to cellulose acetate membranes. Gels were run under conditions described above and stained for GPI activity.

Our experimental design required distinguishing among no more than two potential paternal colonies. Consequently, we employed male colonies that were homozygous for two different alleles. Females were homozygous for one of the same pairs of alleles. Heterozygous offspring brooded by the female were thus concluded to be fathered by the male colony homozygous for the alternate allele, and homozygous offspring by the male that was homozygous for the same allele as the maternal colony. For each male colony in an array, relative fertilization success was initially assayed as the percentage of embryos fathered. Female fertilization success was calculated for each central female colony by determining the percentage of eggs that had developed (quantified by counting the number of unfertilized eggs held by each female prior to deployment and the number of embryos brooded upon recovery). In order to adjust male paternity for variation in the percentage of the female's eggs fertilized by all males, we also calculated a second estimate of male fertilization success according to the following equation:

$$\%\ \text{available eggs fertilized} = \frac{\text{No. embryos} \times \%\ \text{embryos fathered}}{\text{No. eggs initially produced}}.$$

In both spacing-distance experiments, we examined the effect of male density treatments on female fertilization success via isotonic regression, which permits a one-tailed test of a hypothesis that specifies the order of expected results (see Gaines and Rice 1990). Previously published work (Levitan 1991, Levitan et al. 1992) led us to expect a priori that female fertilization success would increase with male density. Percentage values for both female and male fertilization success are reported in the text and figures as raw values, but were arcsine-transformed prior to statistical analysis to normalize distributions.

We examined the effect of competing colonies on both estimates of male fertilization success by performing two different t tests of paired samples in each experiment. For each estimate, we first compared the fertilization success of close and distant males within the competitor-present treatments, and secondly the fertilization success of distant males in the presence and absence of the nearer males (i.e., between treatment comparison of distant male's fertilization success). We also calculated allele and genotype frequencies in our screened population and tested for genotype frequency deviation from Hardy-Weinberg equilibrium with a G test.

Celleporella hyalina

C. hyalina is a cheilostome bryozoan that commonly occurs on fronds of laminarian kelps in the North Atlantic (Osburn 1933, Ryland 1979). Colonies are simultaneous hermaphrodites with gonads confined to specialized unisexual zooids. Male zooids do not feed, but possess a reduced lophophore (Marcus 1938, Hughes 1987) presumed to be employed in the dissemination of sperm (accomplished through the tips of lophophoral tentacles in other cheilostomes; Silén 1972). The internal volume of the male zooid is occupied mostly by spermatogenic tissue (Hughes 1987). Budding of sexual zooids occurs from a basal layer of sterile feeding zooids. Colonies in both Maine and Wales pass through an initial phase in which only male zooids are matured. Subsequently, both male and female zooids are produced and function simultaneously (Cancino and Hughes 1988, McCartney 1994). Colonies in Maine are self-fertile when reared in isolation in the laboratory (McCartney 1994). On both sides of the Atlantic, colonies exhibit extensive variation in the relative abundance of male and female zooids (Hughes and Hughes 1986, Hughes 1989, McCartney 1994); male zooid number in Maine colonies varies over greater than two orders of magnitude (McCartney 1994). Differences in gender allocation appear to be genetically determined and stable when colonies are clonally propagated (Cancino and Hughes 1988, Hughes 1989).

Adult colonies used in mating experiments were reared from larvae by methods modified after Cancino and Hughes (1988). We collected fronds of *Laminaria longicruris* that were heavily encrusted with adult *C. hyalina* colonies from a location near our field site. Fronds were held in running seawater and total darkness for 16 h, then transferred to glass aquaria and illuminated with a fluorescent light source. Release of copious quantities of larvae ensued. Larvae were collected and placed in rectangular tanks containing glass slides held in slide boxes. Circulating flow induced via air bubbles was used to induce rapid settlement onto unconditioned glass surfaces (see McCartney 1994). Following settlement, juvenile colonies were reared in the laboratory in flowing seawater and fed every 2 d with a phytoplankton suspension consisting of equal volumes of *Tetraselmis* sp., *Isochrysis* sp., and *Rhodomonas* sp.

At ≈3–4 wk of age, just prior to expression of sexual maturity under laboratory growth conditions, we screened these colonies for the presence of allozyme markers. We removed small portions of each colony (5–10 zooids) and placed each portion into 10 μL of cold homogenization buffer (10 mL of 0.1 mol/L Tris-HCl pH 7.5, 10 μL Triton X-100 [Sigma], and 10 μL β-mercaptoethanol) in a multiwell grinding plate. Samples were briefly homogenized and held at 4°C prior to electrophoresis. Adult colony homogenates were assayed utilizing electrophoresis techniques as described for *B. schlosseri,* except that we employed 0.025 mol/L Tris–0.192 mol/L glycine pH 8.5 as a running buffer and conducted electrophoresis for 1 h at 200 V. Gels were stained for GPI as described above. *C. hyalina* colonies collected from the Damariscotta River are polymorphic for at least seven GPI alleles. Allele designations are expressed as described for *B. schlosseri.*

Colonies homozygous for alleles GPI-80, GPI-100, and GPI-120 were commonly collected. Individual colonies homozygous for one of these alleles were identified and roughly square sections of the slide on which each was settled were cut out using a glass cutter. Each section was glued onto a standard microscope slide with silicon adhesive and the slides were assembled into mating arrays. Colonies were mounted onto acrylic panels (46 × 15 cm) in two different treatments. The competitor-present treatment housed three colonies in a linear array, with 2.5 cm separating the approximate center of each adjacent colony and the centers aligned on the longitudinal axis of the panel ($n = 6$ replicates; Fig. 1G). Competitor-absent trials employed a similar arrangement of only two colonies with the middle (intervening) position left vacant, so that the two colonies in the end positions were separated by 5 cm ($n = 5$ replicates; Fig. 1F). In both treatments, colonies were positioned at random with respect to genotype.

Arrays were deployed in the field by the methods described above for *B. schlosseri* and positioned on the same grid. The grid was again far removed from the closest population of *C. hyalina,* which encrusts kelp fronds in a bed located ≈0.6 km downriver. No adult colonies were present on the adjacent hard substrata at this site, but juveniles did occasionally settle upon the blocks and panels upon which the arrays were deployed. To limit exogenous sources of sperm, patrolling divers removed these colonies and any drift kelp (potentially carrying *C. hyalina* colonies) that had become lodged on the arrays or grid lines. After ≈2 wk of growth in the field, we counted the sexual zooids in each colony. All slides were removed from mating arrays by a diver and confined as a group in plastic containers to prevent cross-contamination among colonies on different arrays. Containers were transported back to the laboratory and maintained in isolation in flowing seawater.

We enumerated sexual zooids using a method adopted from Hughes and Hughes (1986). Using a camera lucida, we traced the outline of each colony to obtain colony areas. Triangular subsections of each colony were selected at random, and all sexual zooids within these subsections were counted and zooid gender identified. We calculated total colony areas from the tracings using the OPTIMAS static-image package (Bioscan, Edmunds, Washington) available on an image-processing system (Motion Analysis, Santa Rosa, California). Next, we divided total colony area by the proportion of this area that was subsampled for sexual zooids and multiplied this value by the number of sexual zooids counted in the subsampled area to produce an estimate of the total number of male and female zooids on the entire colony. Male zooid counts obtained by this method were used as a measure of male gonad investment for paternal colonies.

After quantifying sexual zooids, we returned the arrays to the field for a 16-d period to allow fertilization to occur. This time interval is slightly longer than the average duration of the brooding cycle for *C. hyalina* colonies in Maine, from time of appearance of a single egg in the zooid body until its release as a mature larva (15.7 d. ± 0.8 d; mean and 95% confidence interval; P. O. Yund and M. McCartney, *unpublished data*). Since embryos were harvested ≈1–2 d prior to their release, fertilization of >95% of the eggs must have occurred soon after redeployment of the arrays. Counts derived from our tracings thus accurately reflect the number of sexual zooids present at the time of fertilization of eggs that developed into harvested embryos.

At the end of this 16-d period, colonies were returned to the laboratory and embryos obtained for paternity analysis. From colonies brooding >24 2-wk-old embryos, we harvested 24 embryos as a subsample, while for colonies brooding ≤24 embryos, we harvested the entire brood. Embryos were obtained by cracking the ovicell using a fine needle and forcing a stream of water over the dissected ovicell to free the embryo. We collected embryos in 1 μL of seawater with an automatic pipetor and transferred each into 2 μL of grinding buffer in the wells of an HLA plate held on ice, in which embryos were homogenized with a blunted needle. Electrophoresis and staining were conducted as described above. Heterozygous embryos were assigned paternity to the single colony on the mating array that carried the paternal allele present in the embryo. We concluded that embryos that were heterozygous for alleles not represented in colonies on the array must have been sired by exogenous sperm sources (contaminants). Finally, embryos homozygous for the maternal allele were inferred to have resulted from self-fertilization.

Each of the field mating trials assessed the ability of the colony positioned at one end of the array to fertilize eggs of the colony positioned at the other end. In the competitor-present treatment, we were able to harvest embryos from each end colony in five of the replicates and from one end colony on the remaining replicate (the maternal colony at the other end position in this replicate produced no female zooids). This yielded 11 end-position females for the competitor-present treatment. The competitor-absent treatment yielded embryos from each of the two end colonies in all five replicates, generating a sample of 10 maternal colonies.

We measured male fertilization success as the proportion of the total brood or the brood subsample that was heterozygous for the paternal allele. We reported untransformed values in the text and in figures, and arcsine-transformed these values prior to statistical analysis (transformed values were normally distributed; Shapiro-Wilk; $W = 0.980$, $P = 0.717$). To assess the proximity-dependence of male fertilization success, we compared paternity values for middle colonies with that of end colonies within the competitor-present treatment with a paired comparisons test (Sokal and Rohlf 1981). Instead of being a function of proximity, the male fertilization success of middle colonies could

be determined by their sperm output relative to that of end colonies. To test this hypothesis, we employed a linear regression to examine the relationship between the proportion of embryos sired by the middle colonies and an estimate of their relative sperm output (number of male zooids in middle colony divided by the sum of the male zooids on both middle and end colonies).

To determine whether fertilization success of end colonies was affected by the presence of middle (intervening) colonies, we compared paternity assigned to end colonies in the competitor-absent trials and the competitor-present trials by one-way ANOVA, with presence/absence of competitor as the fixed treatment effect. We refined this analysis by including number of male zooids in end paternal colonies in the model as a linear covariate, since previous experiments have shown this character to be a reliable determinant of fertilization success in *Celleporella* (McCartney 1994). This ANCOVA model was used to assess the effect of competing colonies on paternal success of end colonies with differences among end colonies in estimated sperm output held constant.

Embryos identical to their maternal parent at the GPI locus were concluded to have resulted from putative self-fertilization events. Since facultative self-fertilization in hermaphroditic animals is thought to occur in response to sperm limitation (Charlesworth and Jarne 1993), we employed linear regression to test for a negative relationship between the proportion of selfed embryos and the availability of outcross sperm (estimated by the total number of male zooids available on all potential outcross paternal parents). Data from maternal colonies in both treatments were combined for this analysis.

Contamination from exogenous sperm

Although the spatial separation of our mating arrays from each other and from natural populations was designed to minimize the presence of sperm from outside the arrays, some contamination is likely to have occurred (see *Results*). Since contaminating sperm can be expected to disproportionately carry more common alleles, their presence might bias the results of our mating experiments by leading to inflated paternity estimates for experimental males possessing more common alleles. Contamination bias in the ascidian experiments was minimized through experimental design, while potential contamination bias in the bryozoan experiments was analyzed post hoc.

We employed different experimental designs in the two different spacing-distance experiments performed with *B. schlosseri.* In the expanded-spacing experiment, half of the replicates within each treatment were assembled with the distant colony homozygous for the rarer allele (GPI-115) and half with the distant colony homozygous for the more common allele (GPI-100). This arrangement was designed to equalize any bias introduced by contaminating sperm between treatments. In the close-spacing experiment, the distant colony in the competitor-absent treatment was homozygous for the rarer allele, thus increasing the probability of detecting contaminants (which would more likely contribute the common allele). In the competitor-present treatment of the close-spacing experiment, the distant colony was homozygous for the more common allele and the nearer colony was homozygous for the rarer allele. This design would have caused contamination to bias the results against the hypothesized effect, as contaminating sperm would have been disproportionately recorded as fertilizations by the distant colony.

In the bryozoan mating experiments, we detected some alleles in progeny that were not included in any of the test colonies on the arrays, indicating the presence of some contaminating sperm. We performed two tests to assess the impact of contaminating sperm on our results: (1) We tested for the possible inflation of paternity estimates for colonies carrying more common alleles by one-way ANOVA with paternal genotype as the fixed treatment factor. (2) To determine whether inflated estimates could confound the ANCOVA analysis of experimental treatments, we performed a two-way ANOVA using genotype and presence/absence of competitor as the two fixed factors. Evaluation of the interaction term in this model provides a test for whether false paternity assignment differed among treatments. We then included male zooid number again as a covariate. Evaluation of the interaction term between the covariate and paternal colony genotype provided a test of whether contamination confounded our analysis of the effect of male zooid number on fertilization success.

Flow regime

Our experimental designs implicitly assume that flow is bidirectional and non-zero at the time of fertilization. To test this assumption, we characterized the flow regime experienced by our mating arrays in the field by deploying an Endeco Type 105 recording current meter (Marion, Massachusetts) at the northeast corner of our grid for a 4-wk period. This analog mechanical meter measures current speed from 0 to 180 cm/s at a threshold of <2.6 cm/s and records direction of flow at a resolution of $\pm 1°$. Time-averaged values for both speed and direction were recorded photographically once every 30 min. These photographic records were subsequently digitized to yield speed and direction data at each point in time. Meter deployment was concurrent with half of the *C. hyalina* replicates and extended 2 wk beyond the termination of these trials. Current measurements over this 4-wk period should adequately characterize the range in tidally driven flow at this site and are intended only to describe the range of conditions under which fertilization is likely to have taken place. Since we do not know exactly when fertilization

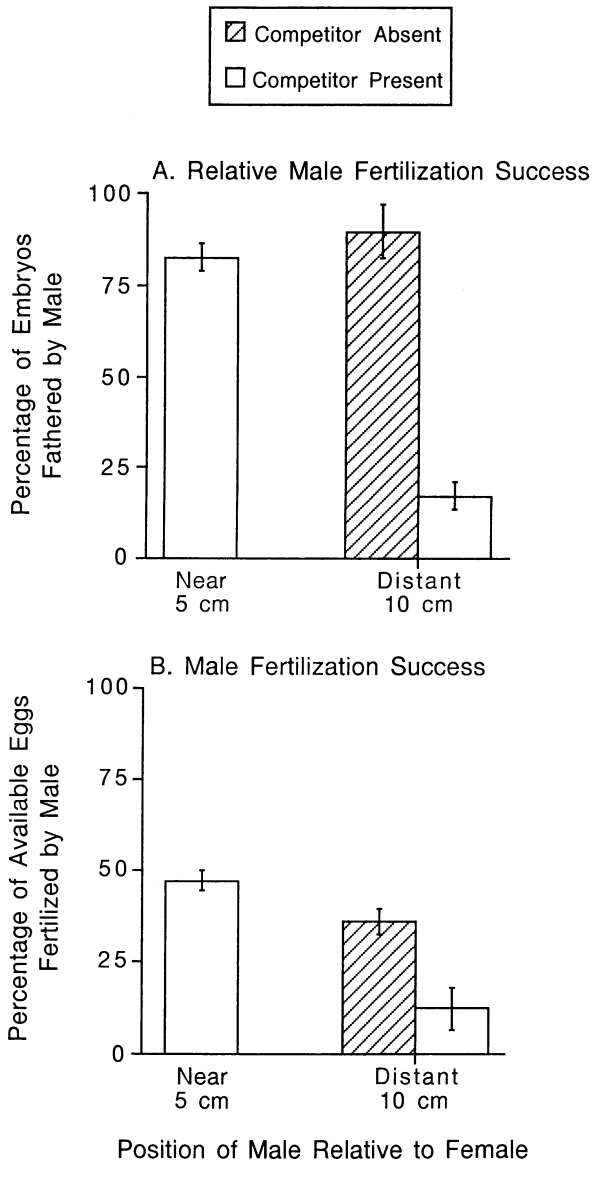

FIG. 2. Two estimates of male fertilization success for *Botryllus schlosseri* colonies in the close spacing-distance experiment. (A) Percentage of embryos fathered by males 5 and 10 cm from the central female colony. (B) Percentage of available eggs fertilized by males 5 and 10 cm from the central female colony. Error bars represent ±1 SE.

occurred, we cannot know the flow velocity at that precise point in time.

RESULTS

Botryllus schlosseri

We screened 303 *B. schlosseri* colonies from the population adjacent to our study site to obtain experimental genotypes. Three GPI alleles were detected with frequencies of 0.68 (GPI-100), 0.30 (GPI-115), and 0.01 (GPI-105). Due to the rarity of the GPI-105 allele, GPI-105 homozygotes were not detected in our relatively small sample. Consequently, only GPI-100 and GPI-115 homozygotes were employed in mating experiments. Genotype frequencies for GPI-100 and GPI-115 bearing colonies did not differ significantly from Hardy-Weinberg expectations (G test, $G = 3.63$, 1 df, $P > 0.05$).

In the close spacing-distance fertilization experiment, female fertilization success increased among treatments with an increasing density of males (isotonic regression, hypothesis: control < competitor-absent < competitor-present, $E^{-2} = 0.447$, $P = 0.003$; means were 25.5, 41.5, and 59.8% of eggs fertilized, respectively). In the competitor-present treatment, nearer males had a significantly higher relative fertilization success (percentage of embryos fathered) than did more distant males (Fig. 2A; t test for paired comparisons, $t = -7.804$, 6 df, $P < 0.0001$). The relative fertilization success of distant males was also dramatically lower in the competitor-present treatment than in the competitor-absent treatment (Fig. 2A; t test for paired comparisons, $t = -7.998$, 6 df, $P < 0.0001$). It is possible that the decrease in relative paternity of distant males might not accurately reflect changes in absolute paternity, because female fertilization success increased between treatments (i.e., the percentage of embryos fathered declined, but more eggs were fertilized in total and hence a similar number of embryos may have been fathered). However, a comparison of the magnitude of change in female and male fertilization success between treatments suggests that the absolute male fertilization success of the distant male did decrease. Relative male fertilization success decreased by a factor of 5.2 between treatments, while female success increased by only a factor of 1.4. Hence the increase in the percentage of eggs fertilized offset only a portion of the decline in relative male fertilization success. To further quantify these differences, our second estimate of male fertilization success (percentage of available eggs fertilized) adjusted relative male fertilization success for variation in the percentage of eggs fertilized by all males. The percentage of available eggs fathered by distant males was significantly lower in the competitor-present treatment (Fig. 2B; t test for paired comparisons, $t = -3.719$, 6 df, $P < 0.01$). These results indicate that males did compete for fertilizations in this experiment.

Female fertilization success also increased significantly among treatments with male density in the expanded-spacing experiment (data for control females are the same as reported above; isotonic regression, hypothesis: control < competitor-absent < competitor-present, $E^{-2} = 0.399$, $P = 0.02$; means are 25.5, 34.5, and 59.7% of eggs fertilized, respectively). There were trends for nearer males to have a higher relative fertilization success than more distant males in the competitor-present treatment and for the relative fertilization success of distant males to be lower in the competitor-present treatment than in the competitor-absent treatment, but neither of these differences were

statistically significant (Fig. 3A; t test for paired comparisons, $t = -0.653$ and -1.561, respectively, 5 df, $P > 0.5$ and $P > 0.15$). Even if the nonsignificant trend toward higher male relative paternity for the distant male in the competitor-absent treatment is treated as reflecting a real biological difference, adjusting the variation in relative paternity by the change in female fertilization success completely eliminates any apparent difference between treatments. The percentage of available eggs fathered by distant males remained constant between treatments (Fig. 3B). Thus, the results from this experiment contrast with those from the close spacing-distance experiment. The presence of an additional male increased the percentage of eggs fertilized, but that increase was not accomplished at the expense of the distant male. Hence no competition for fertilizations occurred in this experiment.

Celleporella hyalina

We screened 2150 adult *C. hyalina* colonies settled from larvae and observed seven electromorphs at the GPI locus. Three relatively common alleles were present at frequencies of 0.509 (GPI-100), 0.254 (GPI-80), and 0.220 (GPI-120). Genotypic frequencies for individuals carrying these alleles did not differ from Hardy-Weinberg expectations (G test, $G = 3.877$, 4 df, $P > 0.25$), and we readily recovered homozygotes of each of these three alleles. Four additional alleles were present at frequencies of 0.0005 (GPI-30), 0.003 (GPI-40), 0.005 (GPI-60), and 0.0079 (GPI-140), and so were too rare to be obtained in homozygous states.

In the competitor-present treatment there was a trend for more progeny to be sired by middle colonies than by end colonies (40 vs. 33%), but this trend was not statistically significant (paired-comparisons analysis; Sokal and Rohlf 1981; $F = 0.424$, $P > 0.5$). Our inability to detect an effect of proximity in this treatment may derive from the fact that we did not experimentally control relative sperm production between middle and end colonies. The proportion of the total (middle and end) number of male zooids formed by middle colonies varied among trials (Fig. 4) and was positively associated with the proportion of embryos sired (Fig. 4; linear regression, $r^2 = 0.43$, $P = 0.039$, with the detected outlier [standardized residual >2 standard deviations] discarded prior to analysis).

As was true for the experiments performed with *B. schlosseri,* comparisons between the competitor-present and competitor-absent treatments indicate that paternal success of more distant *C. hyalina* colonies was diminished when an intervening colony was present. We assessed this effect in two different ways. First, mean relative paternity obtained by end colonies in the competitor-absent treatment was significantly higher than that observed in the competitor-present treatment (means of 0.48 and 0.29, respectively; ANOVA, $F = 5.062$, $P < 0.05$), but only when the trial detected as an outlier in the above analysis was eliminated.

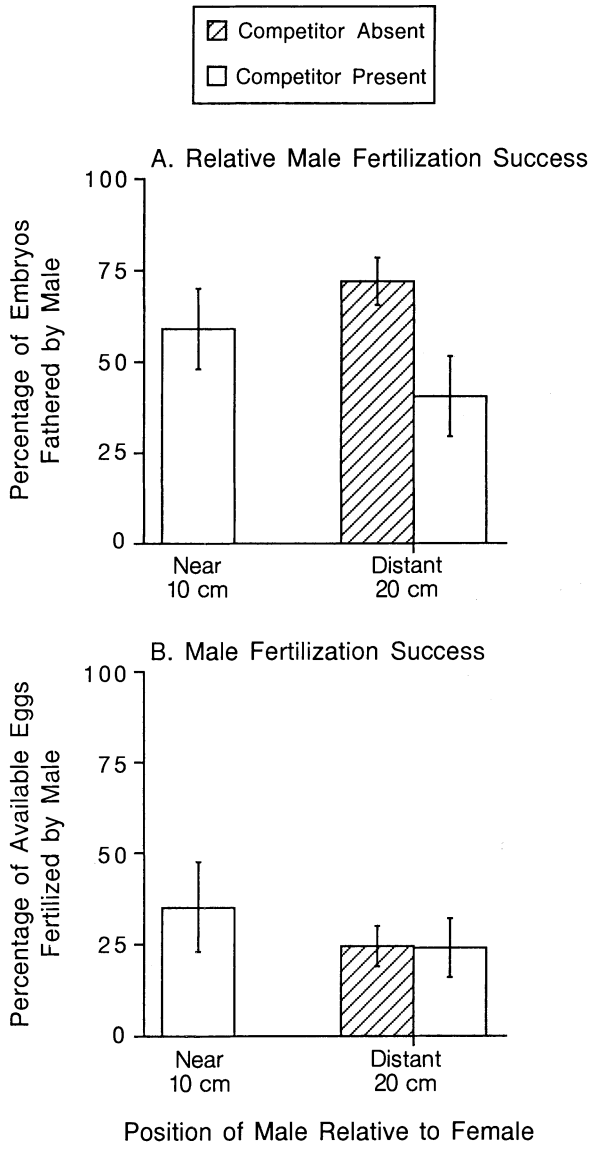

FIG. 3. Two estimates of male fertilization success for *B. schlosseri* colonies in expanded spacing-distance experiment. (A) Percentage of embryos fathered by males 10 and 20 cm from the central female colony. (B) Percentage of available eggs fertilized by males 10 and 20 cm from the central female colony. Error bars represent ±1 SE.

The second, potentially more powerful method of analysis adjusted for differences in male reproductive effort among the paternal colonies. End colonies in the mating arrays displayed a 15-fold range in the number of male zooids present (Fig. 5). Within each treatment, there were significant positive linear relationships between male zooid counts in end colonies and their paternal success estimates (Fig. 5). Consequently, we employed analysis of covariance to test for an effect of treatment when adjusted for variation in male zooid number. With number of male zooids formed by the paternal end colony as a covariate, end colonies in the

FIG. 4. Paternal success of intervening colonies in the competitor-present treatment with *C. hyalina*. Values on the *x* axis represent the proportion of the total number of male zooids counted on potential paternal colonies at both the end and intervening positions that are produced by the intervening colony. The values on the *y* axis denote the proportion of embryos sired by the intervening colony. The fitted line is the least-squares linear regression line computed when the indicated outlier (arrow) is omitted from the analysis. The outlier has a standardized residual >2 standard deviations.

competitor-absent treatment obtained significantly more fertilizations than end colonies in the competitor-present treatment (Table 1). Treatment differences were significant before exclusion of the previously identified outlier ($F = 6.354$, $P = 0.021$), but were highly significant (Table 1) upon its elimination. Assumptions of ANCOVA are upheld; the covariate yields a highly significant regression ($F = 14.96$; $P = 0.001$) and slopes among groups are homogeneous ($F = 2.153$; $P > 0.05$). Adjusted group means (±1 SE) from the ANCOVA indicate that end colony fertilization success (51.2 ± 4.4%) was reduced by ≈50% when a competing colony was present (26.7 ± 4.4%).

A closer examination of the within-treatment effects of variation in zooid number (Fig. 5) reconciles the ANCOVA result with the initial ANOVA analysis. End colonies in the competitor-absent treatment consistently obtained more fertilizations than end colonies in the competitor-present treatment that produced similar numbers of male zooids. However, some colonies in the competitor-present treatment that produced large numbers of male zooids obtained more fertilizations than colonies in the competitor-absent treatment that produced few male zooids. This effect created a large overlap in male success and obscured differences between the treatments in the ANOVA. It also illustrates that increased allocation to the production of male zooids can overcome the loss of fertilizations to competitors.

Most *C. hyalina* colonies brooded some progeny that were homozygous for the maternal allele, and these offspring were designated to have developed from putatively self-fertilized eggs. The proportion of the brood assigned to self-fertilization varied considerably among maternal colonies (Fig. 6). We asked whether the incidence of self-fertilization in *C. hyalina* was correlated with the availability of outcross sperm, as assayed by the total number of male zooids found on outcross paternal colonies. For all maternal colonies pooled, we obtained a significant inverse relationship between the proportion of putatively selfed progeny and the number of outcross male zooids on an array (Fig. 6; linear regression, $F = 7.337$, $r^2 = 0.23$, $P < 0.012$).

Contamination from exogenous sperm

The alleles that we employed as paternity markers were relatively common in natural populations, and so were not necessarily unique to our experimental colonies. Consequently, some of the fertilizations that we have nominally attributed to experimental males might have come from outside our mating arrays. Since contaminating sperm can be expected to disproportionately carry more common alleles, their presence might bias the results of our mating experiments by leading to inflated paternity estimates for experimental males possessing common alleles. We first address the question of whether contaminating sperm were present, and secondly consider whether contamination could have generated the results that we obtained.

Although several lines of evidence suggest that *B. schlosseri* colonies did receive sperm from exogenous sources, different contamination estimates vary in magnitude. Female colonies placed in the field without experimental males present were fertilized at the appreciable rate of 25%. Embryos brooded by these col-

TABLE 1. ANCOVA results for *Celleporella hyalina* mating success as a function of competition treatment and sperm production. Dependent variable is (arcsine-transformed) proportion of embryos sired. The model consists of treatment group (competitor present vs. competitor absent) as the main effect, with number of male zooids on the fertilizing colony as the covariate.

Source	df	Sum of squares	Mean square	*F*	*P*
Treatment group	1	0.357	0.357	15.593	0.0010
Number male zooids	1	0.342	0.342	14.965	0.0012
Error	17	0.389	0.023		

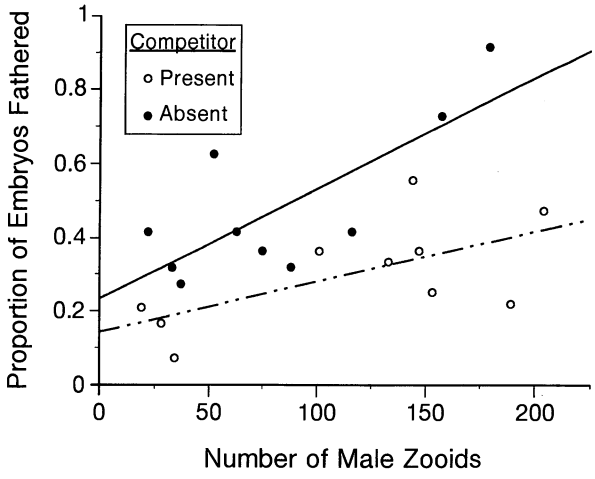

FIG. 5. Paternal success of end colonies of *C. hyalina* in both the competitor-present and competitor-absent treatments. *x*-axis values correspond to the number of male zooids produced by the fertilizing (end) colony; *y*-axis values are the proportion of progeny brooded by the maternal (end) colony that are sired by the fertilizing colony. Symbols denote whether values were obtained from the trials in which competing colonies were present or absent; lines represent within-group regression lines derived from ANCOVA.

onies displayed substantial variation in developmental timing (in contrast with the highly synchronous development of embryos from females from arrays with experimental males present; P. O. Yund and M. A. McCartney, *personal observation*), suggesting that embryos from control females were the product of fertilizations acquired at a low rate over an extended time period. Possible sources of sperm for these matings include experimental males on other arrays (separated by at least 10 m), natural populations at other locations in the estuary (separated by a minimum of 40 m cross-stream), and colonies growing on kelp (*Laminaria* sp.) that frequently drifted through the area after being dislodged from other locations in the estuary. The frequency of paternal alleles in embryos from control females matched the frequency of the alleles in local populations (P. O. Yund and M. A. McCartney, *unpublished data*), suggesting support for both the second and third possibilities.

If 25% of the available eggs in the treatments with experimental males were fertilized by exogenous sperm, noise from contamination might overwhelm the signal from experimental males (since only 35–60% of the available eggs were fertilized) and greatly reduce our ability to detect local fertilization patterns. However, data from the competitor-absent ascidian treatments yield additional, lower estimates of contamination in the experimental treatments. Known contaminating sperm (i.e., not attributable to the single male present on these arrays) accounted for 10% of the embryos fathered in this treatment in the close-spacing experiment (Fig. 2A) and 28% of the embryos fathered in the expanded-spacing experiment (Fig. 3A). These contamination levels correspond to 4 and 10%, respectively, of the available eggs fertilized by exogenous sperm. This decline in contamination levels between treatments is consistent with our original hypothesis that closer males displace sperm from more distant males and suggests that contamination may have been even lower in the competitor-present treatments (for which we lack an independent estimate of contamination, as both common alleles were present in experimental males). Consequently, background fertilization rates of control females are likely to overestimate contamination in arrays with experimental males.

There was also evidence of contaminating sperm in the *C. hyalina* experiment. First, the rare allele GPI-140 was found to occur in embryos harvested from the arrays. Since this allele was not carried by any experimental males, it must have been contributed by contaminant sperm. However, this allele occurred in few of the trials (3 out of 10 of the competitor-present and 1 out of 10 of the competitor-absent trials). Secondly, one of the two alleles belonging to the less common class (GPI-80 and GPI-120) was absent in each replicate array of the competitor-absent treatment, and so the presence of that foreign allele in embryos harvested from those arrays must be due to contamination. The frequency of occurrence of these foreign alleles varied among trials within the competitor-absent treatment (mean of 0.075 with a standard error of 0.024) and occasionally accounted for a large fraction of fertilization events (maximum frequency of 0.45).

Given that some contamination occurred, could it have produced the observed results? The ascidian ex-

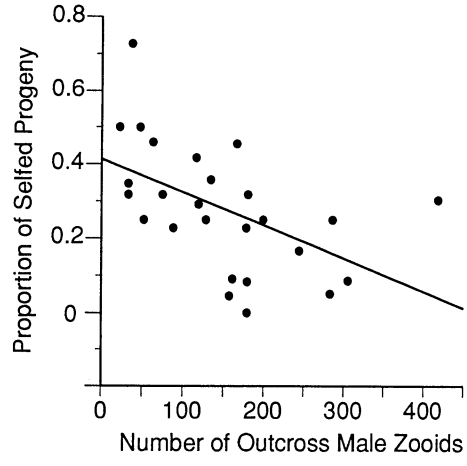

FIG. 6. Incidence of self-fertilization and availability of outcross sperm in *C. hyalina*. Proportion of embryos in all maternal colonies that result from apparent self-fertilization events (*y* axis) are plotted against the total number of male zooids counted on outcross mates available on the mating arrays. The line is the least-squares linear regression line, which is significant: $F = 7.337$; df (regression) = 1, df (residual) = 24; $P = 0.012$.

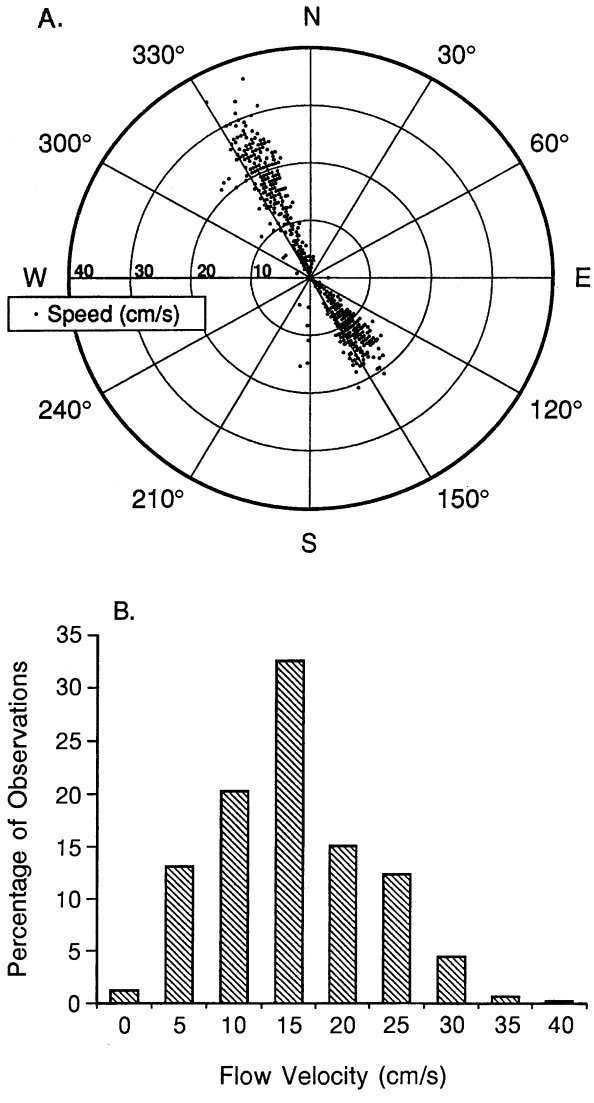

FIG. 7. Flow characterization. (A) Polar plot of a random subset of half the data. Polar axis is compass degrees and radial axis is flow speed (cm/s). (B) Frequency distribution of flow speeds.

periments were designed so that contamination would have either biased the outcome against the results obtained (close-spacing experiment) or equalized bias between treatments (expanded-spacing experiment; see *Materials and methods: Contamination from exogenous sperm*). Consequently, contamination should have had minimal effects on the results obtained for this species.

Contamination could have spuriously generated the results obtained in the bryozoan experiment only if the following criteria had been met. First, colonies possessing more common alleles would have had to receive disproportionately higher paternity assignment. End colonies that were homozygous for GPI-100 did indeed have higher paternal success than colonies homozygous for GPI-80 or GPI-120 (Tukey's hsd, $P < 0.05$ for both pairs of means). Secondly, treatments would have had to differ in the extent to which genotype-dependent paternity was observed or genotype effects would have had to be non-randomly distributed among colonies ranked by numbers of male zooids. However, there was no significant interaction between genotype and treatment (two-way ANOVA; interaction MS = 0.006; $F = 0.194$, $P > 0.80$). When male zooid number was included in the model as a covariate, there was a significant effect of treatment ($F = 9.697$; $P < 0.01$) but not genotype ($F = 2.029$; $P > 0.10$), and male zooid number was a significant covariate ($F = 6.318$; $P < 0.05$). Homogeneity of slopes in this analysis confirms no interaction among the effects of genotype and male zooid number. Consequently, the bias in favor of more common alleles introduced by the presence of contaminating sperm could not have generated our reported results.

Ambient flow

During the almost 4 wk of monitoring current direction and velocity at our study site we obtained a total of 1387 measurements. The direction of flow (mean ± 1 SE) was 141.5 ± 1.73° (south-southeast) on ebbing tides and 330.8 ± 0.35° (north-northwest) on flooding tides (a random subset of these data are presented in Fig. 7A). The difference between these two angles produces a deviation from perfectly bidirectional flow of 9.3°.

The current meter employed in this study records each velocity reading as a value averaged over a 30-min span of continuous impeller operation. Flow velocities obtained at this level of temporal resolution range from 0 to a maximum of nearly 38 cm/s (Fig. 7B). Intermediate flows clearly predominate at this site. In addition, the meter measured 0 velocities (or flow below the threshold of detection) during the entire 30 min period for <2% of the sample intervals (Fig. 7B). Assuming that the meter's method of sampling does not lead to a biased assessment of mean flows and their fluctuations, we thus expect that near-zero flows will occur, on average, during a time window of <15 min/d. Since the typical swimming velocity of invertebrate sperm is <0.01 cm/s (Vogel et al. 1982), transport of sperm released at this site will, through much of the day, be dictated by current-driven advection.

These results indicate that male colonies in our mating arrays were generally positioned upstream and downstream of female colonies, with only brief periods of slack tide. While these data describe the mainstream flow at this site, they do not address microscale flow patterns over the mating array plates. Velocity is likely to have been lower within the boundary layer of the plates. However, the velocity gradient is not likely to have varied among positions on the plates. Laminar-flow velocity profiles over the plates have been measured in a recirculating flume and have shown no dif-

ferences in flow regime among positions within a plate (McCartney 1994).

Discussion

Competition for fertilizations

Closely spaced *B. schlosseri* colonies competed for fertilizations, with males closer to the female acquiring fertilizations at the expense of more distant males (Fig. 2). However, competition for fertilizations was not an inevitable consequence of the presence of two male colonies capable of fertilizing one brood of eggs. When male colonies were smaller and spaced further apart, the two males each fathered a portion of the embryos brooded by the female colony (Fig. 3A). An increase in the percentage of the female's eggs fertilized, however, offset the distant male's apparent (but nonsignificant) loss of fertilizations as assayed by the percentage of embryos fathered. When male fertilization success was assayed as the percentage of available eggs fertilized (a more accurate estimate of absolute male fertilization success), the distant male's success did not change with the addition of a closer male (Fig. 3B).

We also found evidence of competition for fertilizations in *C. hyalina.* End colonies on mating arrays had lower male fertilization success when a middle colony was present (Table 1; Fig. 5). Since there was no apparent variation in female fertilization success in *C. hyalina* (>95% of female zooids in both treatments contained developing embryos; P. O. Yund and M. A. McCartney, *unpublished data*), competition for fertilizations may be unavoidable in this species when more than one male is capable of fertilizing a brood of eggs. However, the negative relationship between self-fertilization rates and the availability of outcross sperm that we observed (Fig. 6) suggests that fertilizations gained by additional males may to some extent simply reduce the number of selfed progeny, rather than subtracting from the fertilization success of other males. Consequently, there may be scenarios (combinations of density and sperm production) in which two or more *C. hyalina* males can acquire as many fertilizations as each would if present alone.

Our results for *C. hyalina* do differ from those for *B. schlosseri* with regard to the importance of relative proximity to a female on male fertilization success. In closely spaced *B. schlosseri* colonies, closer males acquired substantially more fertilizations than distant colonies (Fig. 2). A similar trend was present in the data for *C. hyalina* colonies, but the differences were not statistically significant. Any proximity effect may have simply been masked by variation in relative sperm output of the end and middle colonies, as middle colonies sired embryos in relation to their male gonad production relative to end colonies (Fig. 4). Furthermore, since ovulations are not synchronized across all ovicells in a *C. hyalina* colony, variation in the timing of sperm release could obscure any distance effects. Nevertheless, our evidence for sperm competition in this species argues that a considerable fraction of spawnings were synchronous.

In *B. schlosseri,* we were able to estimate variation in both female and male fertilization success simultaneously. Since ecological processes may have very different effects on these two components of fitness, future studies should incorporate attempts to determine both. In particular, we have shown that male fertilization success is likely to be competitive in nature. The likelihood that male reproduction is competitive while female reproduction is not is thought to have substantial consequences for the evolution of both behavioral mating systems in animals (Bateman 1948, Trivers 1972, Wade and Arnold 1980) and floral mating systems in angiosperms (Charnov 1979, Willson 1979). Similar ideas may prove applicable to free-spawning marine organisms. In addition, some ecological processes may have opposing impacts on male and female fertilization success (as density of males did in this study). In such cases, measures of both absolute and relative paternity and the yield of fertilized eggs will be required in order to assess total reproductive success.

This study was not designed to assess the effect of variation in total population density on male fertilization success, as only male density was manipulated. However, the results do suggest that the probability that males will compete for fertilizations is likely to increase as a function of population density. Although increased competition will reduce the ability of a male to fertilize any given female, the cumulative effect of variation in population density on an individual male's total reproductive success is difficult to predict. As density increases, the number of females with eggs that can potentially be fertilized by each male should also increase. Consequently, reduced fertilization of the eggs of a given female due to increased competition should be offset by an increase in the total number of available eggs held by different females (assuming that sex allocation does not vary with density). Levitan (1991) suggests that elevated population densities of free-spawning sea urchins may compensate for density-dependent inhibition of female fecundity through enhancement of female fertilization success. Although average male fertilization success must logically increase if average female fertilization success increases (since each egg must be fertilized by some male), the variance in male fertilization success among individuals may be very high due to competition and there are likely to be strong positional effects within a population. Consequently, in order to fully address the effects of density on reproductive success, more data are needed on individual male fertilization success and the relationship between individual success and population density.

Previous theoretical (Denny 1988, Denny and Shibata 1989) and empirical (Pennington 1985, Levitan

1991) studies have indicated that egg fertilization rates in the marine environment may be very low under certain conditions, especially in highly turbulent flow regimes and at low population densities. These observations have led to a view of sperm as the limiting factor in marine fertilization and consequently to an emphasis on the maximization of egg fertilization rates as the focus of natural selection on reproductive traits (Levitan 1993). In contrast, our demonstration of competition among males for fertilizations suggests that under other conditions, sperm may not be in limited supply and selection pressures could instead be exerted on male reproductive traits to enhance performance in competitive situations. Our perspective is more likely to be valid for organisms living in laminar and/or low velocity flows and at high population densities and may be more relevant to brooders than to broadcast spawners. In addition, these two different perspectives may not be mutually exclusive. Competition for fertilizations occurred in our study even when female fertilization success was well below 100% (60% maximum observed in *B. schlosseri*). Note, however, that our assays of fertilization are based on successful embryo development, which could underestimate actual egg fertilization rates in all treatments if developmental failures or selective embryo abortions occur.

Our result showing a reduction in the fertilization success of a male in the presence of an intervening male suggests that gene flow via the dispersal of fertilizing sperm may be impacted by population density. As male density increases, fertilizations should be predominantly obtained by nearer males if sperm competition is occurring (unless near neighbors are gametically incompatible close relatives; Grosberg 1987). As a consequence, the dispersal distance of fertilizing sperm may decrease as population density increases, resulting in reduced gene flow via sperm dispersal. The unidirectional dispersal distance of fertilizing sperm has previously been suggested to be a poor indicator of levels of gene flow because the number of available eggs may increase with distance from a sperm source due to an exponential increase in area with increasing radial distance (Grosberg 1991). However, this observation is only valid if sperm have an equal probability of dispersing in all directions. This is likely not the case in areas like our study site that are dominated by bidirectional tidal flow (Fig. 7).

Reproductive traits and male fertilization success

In *C. hyalina,* male fertilization success on our competitive mating arrays was highly correlated with the number of male zooids produced by colonies (Table 1; Figs. 4 and 5). A similar relationship has been described using different experimental designs at another field site (McCartney 1994), and so is likely to be a general feature of the biology of this species. Colonies with elevated investments in male zooid formation are likely to produce more sperm and hence be capable of numerically displacing sperm released at the same time by colonies with more meager investments. Indeed, colonies in a competitive situation with large numbers of male zooids were able to obtain more fertilizations than colonies in a less competitive scenario with fewer male zooids. In addition, an increased number of male zooids may permit gamete release to be extended over a longer period of time if release by different male zooids within a colony is not simultaneous. An increase in the duration of sperm release might have a major impact on male fertilization success in a species like *C. hyalina,* in which ovulations are not synchronized among zooids in a colony.

Free-spawning marine organisms often allocate much of their biomass to male gonad production (e.g., Loosanoff 1969, Menge 1975). This strategy has previously been viewed either as an evolutionary response to the low efficiency of egg fertilization in the marine realm (Giese and Kanatani 1987) or as a result of selective pressures generated by competition among males for fertilizations (Warner and Robertson 1978). Our results support the latter interpretation.

The field of marine fertilization ecology (this study included) has to date focused mainly on factors influencing fertilization success that are features of populations and are largely beyond an organism's control (e.g., population density, proximity, and flow regime; but see Levitan 1993). While these studies have yielded valuable information on the factors that impact fertilization success in natural populations, progress on understanding how fertilization processes impact the evolution of life history traits would benefit from considerations of the traits and strategies that individuals may use to augment individual fertilization success.

Proposed mechanism of competition for fertilizations

Although we have not attempted to elucidate the processes responsible for sperm competition as demonstrated in this paper, we suggest the following explanation. Evidence from numerous in vitro studies indicates that egg fertilization rates are highly dependent on sperm concentration (e.g., Schmell et al. 1977, Rosati and DeSantis 1978, Lambert and Lambert 1981). In the field, a spawning male produces a plume of sperm that moves downstream. Sperm concentration decreases with distance from both the point of release and the cross-stream axis of the plume (Denny 1988, Denny and Shibata 1989). When multiple males release sperm, these plumes have the potential to overlap. We suggest that the probability of a male obtaining a fertilization at a given location is a function not only of the absolute concentration of that male's sperm, but also of its concentration relative to that of other males. When sperm plumes overlap, any male whose sperm is at a lower relative concentration at a given point will thus have a reduced probability of fertilizing an egg at

that location, even if in the absence of other males he might have a very high probability of fertilizing the same egg. Thus a closer male has a higher relative sperm concentration and hence a higher probability of fertilizing eggs than does a more distant male, and the presence of this closer male may reduce the relative sperm concentration of the more distant male and hence his fertilization success (Figs. 2 and 5). Likewise, a bryozoan colony that allocates more energy to male reproduction may release more sperm, producing a higher relative sperm concentration and leading to higher fertilization success than that of a colony with lower allocation (Table 1; Figs. 4 and 5). Although this model implicitly assumes that males spawn synchronously, the same concept can be expanded to incorporate time integrated variation in relative sperm concentration.

It is likely that sperm plumes will only overlap when sperm are released at high concentrations and the points of release are fairly close together. When *B. schlosseri* colonies were smaller and spaced 10 cm apart, nearer males did not obtain significantly more fertilizations than distant males, and the presence of the nearer male had no effect on the distant male's fertilization success (Fig. 3). At low population densities, male fertilization success may be independent of the presence of other males. The threshold density for interference among males is likely to vary both within and among species as a function of flow conditions and the volume and concentration of sperm release.

Selfing

Previous laboratory studies (Milkman 1967, Sabbadin 1971) have indicated that the colony-wide synchronization of reproductive schedules in *B. schlosseri,* coupled with the temporal separation of ovulation and sperm release, is apparently effective at preventing self-fertilization. We found no evidence of self-fertilization in control females placed alone on arrays in the field. Paternal allele frequencies in embryos brooded by these colonies did not differ from frequencies in a nearby natural population (P. O. Yund and M. A. MCartney, *unpublished data*), suggesting that eggs in isolated colonies were fertilized by a random sample of "background" sperm. In contrast, self-fertilization appears possible in *C. hyalina* due to the overlap in function of male and female zooids through most of the reproductive life of a colony. Recent laboratory studies (Hunter and Hughes 1993, McCartney 1994) have demonstrated that colonies kept isolated from external sources of sperm do produce viable embryos. Colonies in one study (Hunter and Hughes 1993) displayed a reduced level of brooding success, but it is not clear whether this reduction was due to sperm limitation or inbreeding depression.

Our current results suggest that self-fertilization may also occur in *C. hyalina* under field conditions. The proportion of putatively self-fertilized embryos per brood was negatively associated with our measure of the availability of outcross sperm (Fig. 6). This relationship is consistent with the hypothesis that colonies self-fertilize a greater proportion of their embryos when outcross sperm is in limited supply.

Reproductive assurance through self-fertilization has been suggested as a selective factor in the maintenance of self-fertility in both plants and animals (Piper et al. 1986, Charlesworth and Jarne 1993). However, little information is available on selfing rates in natural populations (Charlesworth and Jarne 1993), so the relationship between selfing rate and pollen or sperm limitation remains largely unexplored (but see Karoly 1992). The inverse relationship between self-fertilization and outcross sperm availability in our experimental arrays, though obtained under specific experimental conditions, does suggest that *C. hyalina* colonies may utilize selfing to ensure fertilization when outcross sperm is not available. In nature, this situation is likely to arise under conditions of isolation or low population density.

Conclusions

Since we found evidence of competition for fertilizations in two very distantly related species, the phenomena that we have demonstrated here may well be a common feature of the reproductive biology of sessile and sedentary marine species that live at moderate population densities. The distances between colonies in our experiments were well within the range relevant for natural populations, where colonies frequently settle adjacent to one another and grow into contact (Cancino 1986, Grosberg 1987, Rinkevich and Weissman 1987*a, b,* McCartney 1994). Consequently, competition for fertilizations is likely to be a feature of natural populations. In taxa such as these, the assessment of individual male reproductive success will require information on the distribution, abundance, and reproductive output of other males in a population. Estimates of male fertilization success based on fertilization patterns around isolated males (Pennington 1985, Yund 1990, Levitan 1991, Babcock and Mundy 1992) are likely to overestimate the true performance of males in natural populations.

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