an approach has both advantages and pitfalls; these must be evaluated before any large-scale attempt to 'bank' total genetic information. Ulysses Seal (Captive Breeding Specialist Group, Apple Valley, MN, USA) gave a final cautionary talk on the everyday practicalities of attempting to save individual threatened species. Human population increases, accelerating habitat loss and a lack of relevant information make saving entire ecosystems – or resurrecting them from the frozen state – a very difficult task. Seal urged the participants not to give up hope, but to understand the magnitude of the crisis on many different levels.

The final session was an attempt by the participants to reach some recommendations worth exploring by various conservation biologists. All of the participants noted how little is actually known regarding complex ecosystems. The standard focus in conservation biology has been on saving individual threatened species, for political as well as practical reasons. This approach totally ignores the broad diversity of organisms present in an ecosystem, all of which provide a support system for such threatened animals as pandas or Siberian tigers. Such a support system may be highly resilient to habitat destruction and environmental degradation, or various members of such communities may be exquisitely sensitive. Obviously, a multidisciplinary approach, bringing together microbiologists, geneticists, industrialists, ecologists, conservation biologists, economists, politicians – and a leavening of other disciplines – will be necessary to address such a large and important task.

The participants agreed that a good first step would be to introduce the ideas discussed in the workshop into various existing biodiversity preservation programs. This 'bootstrapping' approach would require modest commitments of time, and bring diverse viewpoints – and perhaps improved strategies – into conservation biology.

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Sperm limitation in the sea

Don R. Levitan and Chris Petersen

Because sperm outnumber eggs, it is often assumed that variation in female reproductive success has little to do with male or sperm availability. Similarly for males, access to viable eggs and sperm competition are thought to drive variation in male fertilization success. These assumptions result from empirical studies on organisms with internal fertilization. However, recent evidence from free-spawning organisms suggests that sperm can often be limiting. This finding may alter our perspective on mating-system evolution, especially in externally fertilizing organisms.

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The concept that males or sperm compete for eggs has shaped our view of mating-system evolution[1]. In animals with internal fertilization, male–male competition explains many patterns of allocation, mating behavior and reproductive morphology[2], but when gametes are released into the environment – the ancestral condition[1] – turbulent diffusion can reduce gamete concentrations to the point where sperm–egg collisions are rare. In such free-spawning taxa[3], availability of sperm may affect female reproductive success. Here, we review the recent evidence for sperm limitation and point out how it can explain patterns of spawning behavior, reproductive allocation and gamete attributes found in marine organisms, and perhaps also provide insight into the evolution of sexual dimorphism.

Evidence of sperm limitation

Despite long-standing speculation[3], Pennington[4] was the first to demonstrate empirically that sperm availability could limit female zygote production. In the 10 years since Pennington's study, observations from natural spawnings, field experiments and theoretical models all suggest that sperm limitation is a regular occurrence.

Observations from natural spawnings

Data collected during natural spawning events of invertebrates (gorgonians[5], sea cucumbers[6,7], corals[8]), fish[9–11] and algae[12] show high variance among females in the proportion of eggs fertilized, often from 0 to 100% within a single species. The lowest fertilization rates (<25%) are those of females that spawn at low population density, in isolation or out of synchrony[3]; females spawning near males (<1 m away) or in synchrony have higher success[3] (>75%). When individuals spawn synchronously in extremely close contact (<1 cm apart), as in the spawning rushes of tropical reef fish[9–11], dense thickets of benthic algae[12] or tight aggregations of invertebrates (>40 sea cucumbers per m^2)[7], average fertilization can exceed 90%, but even then, not all eggs were fertilized and some females had no eggs fertilized.

Experiments

Fertilization rates are influenced by the distribution and size of sperm sources, the rate of sperm diffusion and the dynamics of gamete interactions. Sperm release is a function of biological attributes (individual reproductive effort, body size, spawning behavior), and sperm diffusion is generally a function of the environment (water depth, velocity, turbulence)[3] and spawn consistency[13]. Gamete interactions are controlled, in addition, by attributes like egg size, sperm swimming ability, and how likely a sperm–egg collision is to result in fertilization[3].

Field experiments indicate that female fertilization success (percentage of eggs fertilized) increases with proximity to a male[4,14–19], population density[17,19], population size[19] and sperm output[16,18], and decreases with current velocity[4,19,20]. It tends to drop below 10–20% at >1 m directly downstream from a spawning male (but see Ref. 18), or if only a few scattered individuals are spawning[3]. However, as noted above,

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even when individuals are closely spaced, female fertilization success typically varies greatly among replicate trials[20], suggesting a patchy distribution of gametes in nature. Although experimental studies of fertilization may introduce artifacts that alter the absolute levels of fertilization (see review[3]), the findings concerning the relative rates of fertilization within a study and the high variance in fertilization among replicates should be independent of these artifacts.

In species that release sperm but retain eggs, maternity can be established, and genetic markers can be used effectively to assess paternity and thus track the success of specific males[15,16]. The distance sperm disperse before encountering an unfertilized egg decreases as the number of females increases[15,16], but as the number of individuals increases, so does the number of females that receive sperm[14]. Thus, as the number of spawning individuals increases, neighborhood size decreases (as has previously been noted in plants[21]). In sessile hermaphroditic species, sperm competition increases and either sperm limitation or self-fertilization decreases (dependent on the ability to self) with the number of spawning males[16].

Theoretical models

Theoretical predictions of fertilization success are based on the probability of sperm–egg encounters and on interaction of these gametes to form zygotes. Such studies provide a mechanistic framework for understanding observed rates of fertilization and a basis for predicting how selection might operate on specific adult and gamete attributes. Fertilization rates are influenced by gamete parameters, such as quality and lifespan, egg size and sperm swimming velocity[3,22–24].

Denny and his colleagues[25–27] have modelled gamete dispersal and the probability of fertilization in high-energy environments, such as wave-swept shores. They estimate sperm–egg encounters from release rate and turbulent diffusion of sperm. Although the persistence of sperm tails and motility even in species inhabiting turbulent environments suggests that sperm behavior still plays a role in fertilization, these models assume that turbulence renders sperm motility irrelevant, and thus, that sperm–egg encounters depend only on turbulent diffusion rate of sperm and egg cross-sectional area (target size).

Their results suggest that, in wave-swept environments, the probability of fertilization can be $<1\%$. Under these conditions, increases in population density should have little influence[26], except in surge channels or bays in which water exchange is reduced[27]. Predicted fertilization success under subtidal conditions is much higher because of lower water flow[18,20,28].

Consequences of sperm limitation

The most obvious consequence of sperm limitation is that producing an egg is not equivalent to producing a zygote. This consequence influences both population dynamics and natural selection on characters correlated with fertilization success. Some of the effects of sperm limitation may be counter-intuitive; for example, factors that increase egg production may be correlated with decreased fertilization rates, changing the qualitative relationship between egg and offspring production. Increases in population density can lead to increased intraspecific competition, food limitation and decreased egg production, but, as noted above, they can also result in higher female fertilization success. Quantitative analysis in the Caribbean sea urchin *Diadema antillarum*[17] revealed that an order-of-magnitude decrease in per-capita egg production at high population density was offset by an increase in fertilization success; per-capita offspring production was similar across densities. Gamete production and fertilization might also be inversely related in filter-feeding organisms that broadcast gametes. Increased water movement can result in increased prey capture, body size and gamete production[29], but it also reduces the probability of fertilization[4,10,19,20,26].

The influence of fertilization on population dynamics is mediated by the effect of recruitment on population size. If larval population dynamics are largely density independent, and adult populations are recruitment limited, then sperm limitation would directly affect population size, but if the populations are regulated by severe density-dependent mortality, then the influence of variation in fertilization and recruitment on adult population size would be buffered.

When species are sedentary and larval transport is minimal, sperm dispersal can significantly influence gene flow. Allozyme markers have been used to document the distance over which a male sired offspring in a dense population of the clonal ascidian *Botryllus schlosseri*[15]. These distances (mean = 17 cm) are similar to those over which larvae are transported (mean = 24 cm). Both distances were calculated from the movement of rare alleles from an experimental individual placed in a population. In another study of *B. schlosseri*[16], sperm estimated to be from colonies 40 m from an isolated experimental location fertilized focal females in controls (male-free trials), indicating that, when nearby males are not present to swamp females with sperm, sperm-mediated gene flow may far exceed larval transport. In taxa where larval dispersal is much greater (10s to 1000s of kilometers), gene flow via sperm would have negligible impact on genetic structure.

Reproductive behavior

Spawning synchrony can increase fertilization success. In a Great Barrier Reef coral *Montipora digitata*[8], female fertilization success is highest at the peak of spawning and much lower just before or after or during less-intense spawning events. Aside from selective advantages like predator satiation and optimal use of water currents, spawning synchronization has clear fertilization advantages.

These selective advantages and those of large aggregations should depend on the abundance and mobility of the organisms, as well as the water movement in their environments[20]. Selective pressure for mass spawning should be most intense on sessile or rare organisms[20,30]. When a single male can insure a high rate of fertilization, and the increase in fertilization due to the presence of other males is slight, as in highly mobile tropical fish[9,10], large spawning aggregations may occur for other reasons[31].

When, for any reason, the density of a species falls well below its normal level, and selective pressure to spawn synchronously was previously weak, the lower population size can result in lowered fertilization rates and retard the recovery of the population. For example, the population density of *D. antillarum* was reduced by 99% throughout its range in the winter of 1983–1984. The resulting low levels of spawning synchrony and aggregation[30] have been shown experimentally to result in levels of fertilization much lower than those predicted in previous, high-density populations[17]. This decrease may partially explain the present drastic reduction in larval settlement throughout most of the Caribbean.

Investment in gonads and gametes

The relationship between gamete production and zygote production can influence the evolution of allocation patterns in both hermaphroditic and gonochoric species[16,32].

At low fertilization rates, the near-linear relationship between sperm production and male reproductive success should, all else being equal, select for increased individual investment in sperm production. The argument has also been made, however, that increased sperm competition should also result in increased male allocation[1]. Distinguishing between these two explanations requires examination of spawning conditions and fertilization rates. The high fertilization rates in highly synchronized pair-spawning tropical reef fishes suggest that, in these species, sperm competition is a much more important force than low fertilization rate in selecting for increased male gonadal investment; the same cannot be said for sessile or sedentary marine invertebrates with lower and more variable rates of fertilization.

Among hermaphroditic sea basses, fertilization rates are high (>90%; Petersen and Levitan, unpublished data), and species with similar spawning behavior but higher levels of sperm competition had higher male allocation. However, species that behaviorally limited the diffusion of the released gamete cloud had a lower male allocation than those species that released gametes in a rapid spawning rush, which suggests the additional importance of gamete dilution. Across all species of sea basses examined, male-allocation patterns could best be explained by both sperm competition and behaviors that changed the mixing and dilution of gamete clouds[32].

In addition to sperm competition and spawning behavior, sperm release in one tropical reef fish has been shown to vary with fecundity or size of the spawning female[11].

Invertebrate species that release both eggs and sperm tend to invest equally in male and female gamete production[33]. The demonstrated low levels of fertilization in many of these species imply that equal investment is a function of sperm limitation. In species that retain eggs, however, sperm collection and storage can decrease the likelihood of sperm limitation and yield much higher fertilization rates[15,16]. In these cases, male allocation patterns may change as a function of sperm competition[16]. Unequal investment in male and female gamete production should be most evident in species, such as pair-spawning fish[32], that have high female fertilization rates and low levels of sperm competition.

Low mean female fertilization success, because it equally discounts current and future female sexual reproduction, may not affect female allocation in strictly sexual species, but in species with asexual reproduction, such as budding or fragmentation in many clonal marine invertebrates, low mean fertilization success should select for increased investment in asexual reproduction. Because sperm competition effects are frequency dependent, the effects of mean fertilization success on the ratio of asexual to male investment are less clear.

Gamete attributes

In animals, life history theory has emphasized the dominant, longer-lived diploid phase and has generally dealt in the haploid phase only with the trade-off between investment per egg and fecundity[34], but the same conclusions should apply to sperm[1,2,24]. More generally, factors affecting individual lifespan and energy allocation should apply to gametes just as to the adult, diploid phase. Some examples follow that illustrate the importance of gamete dynamics in the sea.

Life history models attempting to explain variation in egg size among marine invertebrates have focused on selective pressure from planktonic mortality and development times. However, these models have been unable to explain how co-occurring species that develop in the same planktonic environment can have different egg sizes. If sperm limitation and variation in fertilization rates are incorporated into these models, species-specific egg sizes can be selected, because each species can differ in adult population parameters, spawning behavior and flow conditions[24].

Off the west coast of North America, three coexisting species (genus *Strongylocentrotus*) span a fivefold difference in egg size; egg size is inversely related to sperm velocity, and sperm velocity is inversely related to sperm longevity[24]. Laboratory experiments indicate that larger eggs are more likely to be fertilized at a given sperm concentration. Models of optimal egg size indicate that, when sperm availability decreases, selection should favor increases in egg size. High sperm availability would select for smaller eggs and also faster sperm because sperm competition would be more likely. When sperm are less abundant, increased sperm longevity would increase the chance of a sperm's surviving until it encounters an egg. As predicted, the *Strongylocentrotus* species with the smallest egg and fastest sperm inhabits shallow water and tide pools, where sperm concentration is likely to be high, and the species with the largest egg and longest-lived sperm lives in deeper water, at low population density, where sperm concentration is likely to be low[24].

In the deep sea, the lack of environmental spawning cues and low density might produce strong selective pressure for gamete attributes that increase the probability of fertilization by chance encounter. As predicted, deep-sea echinoids tend to have extremely long-lived sperm and larger eggs than shallow-water species[35].

In contrast, tropical reef fish release gametes in close contact, most fertilization occurs within seconds, and sperm are unlikely to encounter eggs after gametes disperse. Sperm competition can also be intense when small males (streakers) join pair spawns or when males group-spawn. These factors should select for immediately active, short-lived sperm, and, in at least one species that meets all the above criteria, sperm appear to be active for only 10–15 seconds[9]. These examples suggest that fertilization dynamics can influence both egg provisioning and sperm energy allocation.

Sperm limitation, sperm competition and sexual dimorphism

That sperm can be limiting in marine systems does not mean that sperm never compete there; several studies[9,16] suggest that sperm limitation and competition form the extremes of a continuum of sperm abundance and can overlap spatially or temporally within the same species or even within a cohort of sibling eggs (e.g. when several sperm contact one egg but miss a nearby egg completely). In patchily distributed sedentary organisms, local conditions are likely to determine whether competition or limitation occurs[36]. Even for mobile organisms, temporal variation in flow and population parameters can result in variation in sperm abundance and produce this result.

Whether sperm compete for virgin eggs or eggs for unattached sperm depends on the ratio of gametes. If eggs are retained in the female and sperm can be collected and perhaps stored (e.g. by ascidians[37]), the relatively abundant sperm may compete for eggs. When eggs are released into the environment, even though total sperm still outnumber eggs by several orders of magnitude, local patches in sperm–egg ratios, and thus the degree of sperm or even egg competition, can vary. Once the gametes are mixed into the water column, available data suggest that fertilization is dominated by factors governing chance encounter of dilute gametes, and not by competition.

Reducing sperm competition and limitation to local sperm and egg concentration provides insight into the general mechanism underlying Bateman's principle – the idea that gender-specific differences exist in the functional relationship between mating success and production of offspring[38], and provide the basis of sexual selection and thus sexual dimorphism. At the level of gamete production, sperm greatly outnumber eggs, but at the point of fertilization, where gamete competition occurs, sperm–egg ratios can be reduced and more variable. When sperm are delivered to eggs internally, or directly onto a batch of eggs, sperm are in relatively high numbers, fertilization is likely with relatively little investment, competition among males is probable, and a high degree of sexual dimorphism (in sex allocation and secondary sexual characteristics) is expected. When sperm or pollen is released into the environment and collected by females, as in higher plants and many marine invertebrates, sperm competition and limitation would be more variable, and a lesser degree of sexual polymorphism is expected. In the case of free-spawning invertebrates, local sperm concentration can be relatively low and highly variable, sperm limitation may be more common than competition, and diminished sexual dimorphism is expected. In addition, in many free-spawning marine fishes, the ability of males to restrict sperm competition is limited, and many males may commonly release sperm in the water column to fertilize a batch of eggs, leading to a second evolutionary pathway with high male allocation, similar near-linear returns on gametic investment for males and females and a convergence of male and female life histories. In this light, it is not surprising that plants tend to have lower sexual dimorphism than do animals[36], and that free-spawning invertebrates tend to have little or no differences in secondary sexual characteristics and equal investment in gonadal tissue[33].

Although pollen limitation appears to occur with some frequency[39], sperm limitation in free spawners may be more common. Aspects of plant biology, such as pollinators, selfing, resorption of unfertilized ovules and accumulation of pollen, may reduce the incidence and costs of pollen limitation. Pollinators have not been observed in marine systems[33]. Selfing does occur in some monoecious invertebrates, and, in these cases, sperm availability may not limit reproduction[16]. Resorption of gametes[5] and sperm storage[37] may be more common in (and perhaps exclusive to) species that retain eggs. In general, higher plants retain ovules for fertilization, whereas marine organisms either retain or release eggs. The fates of released eggs are independent and thus more variable than those of retained eggs. Releasing eggs removes limitations of parental care and brood space, but adults releasing gametes cannot guarantee successful fertilization or recall unfertilized eggs for a second try, and they cannot recoup the energetic cost of unfertilized eggs. In free-spawning taxa, both males and females enter into a fertilization lottery, and both exhibit variation in fertilization success. This situation can have important consequences for the dynamics of populations and the evolution of mating strategies. By seeking to understand the rich diversity of marine mating strategies, we may gain a general understanding of the more constrained strategies noted in terrestrial organisms.

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