

Allocation to Mate Guarding or Increased Sperm Production in a Mediterranean Wrasse

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ABSTRACT: An excellent body of literature exists that examines sperm expenditure when males allocate only to sperm production. However, in many species, males can also allocate energy to behaviors that influence sperm competition. We model whether males in sperm competition should allocate energy to mate guarding or additional sperm production. Mate guarding is predicted to lead to greater reproductive success than increased sperm output, and mate-guarding males are not predicted to alter their allocation to sperm production with increasing sperm competition. Only when mate guarding is ineffective or greatly reduces sperm production are males predicted to allocate to sperm production. In a Mediterranean wrasse *Symphodus ocellatus*, three male alternative reproductive behaviors coexist. While nesting males and satellites guard mates to decrease sperm competition, sneaker males only compete via sperm production. Sneakers produce four times as much sperm per spawn as either nesting males or satellites. As predicted by the model, mate guarding but not sperm production increased with increased risk of sperm competition in nesting males. We argue that this can be explained by nesting males allocating to mate guarding rather than sperm production. Considering allocation among behaviors that affect sperm competition enhances our ability to explain and to predict sperm allocation patterns.

Keywords: sperm competition, energy allocation model, alternative behaviors, energetic trade-off, Labridae.

In recent years, researchers have realized that sperm production is neither cheap nor unlimited in most species. The realization that sperm costs energy and that males may exhibit sperm economy has stimulated extensive research examining sperm competition and allocation (for

reviews, see Birkhead and Møller 1998). Theoretical models have shown that the number of males involved in a mating, as well as the information and energy available to males, can affect their sperm allocation strategy (e.g., Parker 1970, 1990a, 1990b, 1992; Ball and Parker 1996, 1997, 1998a, 1998b; Parker et al. 1996, 1997; Shapiro and Girardeau 1996). For example, game theoretical models predict that, within a species, males should increase the amount of sperm they release when they are in competition with a single other male but decrease expenditure when competing with two or more males (Parker et al. 1996; Ball and Parker 1997). Sperm expenditure is also predicted to increase with increased risk of sperm competition both within and between species (Parker et al. 1997). Other models have examined the effect of behavioral roles on sperm expenditure (e.g., Parker 1990a, 1990b). For example, mate-guarding males are predicted to produce less sperm than males that are always in sperm competition if sperm is equal in cost and achieving paternity (Parker 1990b). However, these models do not consider that males may benefit from allocating energy to other reproductive behaviors, such as mate guarding, that may affect the risk or intensity of sperm competition.

Comparisons across species indicate that sperm production tends to increase with the number of ova per mating and the average level of sperm competition in the species (Birkhead and Møller 1992, 1998; Parker 1992; Gage 1994; Stockley et al. 1996b, 1997). Intraspecific comparisons have shown that males may differentially allocate sperm to females of different fecundities or qualities (e.g., Shapiro et al. 1994; Marconato et al. 1995; Marconato and Shapiro 1996). Individual mating rate may also drive between-male variation in sperm produced per spawn and fertilization rates (Shapiro et al. 1994; Marconato et al. 1995; Warner et al. 1995; Marconato and Shapiro 1996; Warner 1997; Rasotto and Shapiro 1998), and male mating strategy has been shown to affect sperm expenditure (Gage 1994, 1995; Gage et al. 1995; Stockley et al. 1996a).

An excellent body of literature exists examining sperm expenditure patterns when males allocate sperm between matings or allocate energy only to sperm production. And

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in many species, males compete mainly through sperm competition. However, in other species, males may also allocate energy to behaviors, such as mate guarding, that influence sperm competition. A male with limited energy might achieve higher success by allocating energy to a behavior that reduces sperm competition rather than simply producing additional sperm. For example, when given additional energy, sperm-limited males in the bluehead wrasse were found to increase aggression toward sneaker males rather than sperm production (Warner et al. 1995; Warner 1997). Here, we consider a simple model of energy allocation between mate guarding and sperm production. Using the model, we make predictions about differences between male tactics in sperm production, as well as within a tactic in response to the risk or intensity of sperm competition. We then compare the model predictions with field observations of sperm production in a demersally spawning fish. Considering resource allocation among behaviors that affect sperm competition should increase our ability to understand patterns of sperm allocation.

In a Mediterranean wrasse *Symphodus ocellatus*, large males build nests, out of algae, in which all mating occurs. These males also court females and care for the eggs by defending them against predation (Gerbe 1864; Soljan 1930; Taborsky et al. 1987). undefended eggs have no chance of survival (van den Berghe et al. 1989), and about one-third of nests are deserted by the parental male (Taborsky et al. 1987). Past success of the nest seems to determine the probability of desertion, and nesting male success varies greatly between days and between nest cycles (Wernerus 1988; Wernerus et al. 1989). Other males in the population hover around active nests and attempt to join the nesting male's spawns by sneaking (Lejeune 1985; Taborsky et al. 1987). Nesting males, in turn, attempt to reduce the access of the peripheral males to visiting females through active aggression; this is a form of mate guarding (van den Berghe et al. 1989). Larger males dominate smaller males in aggressive interactions, which usually consist of chases (Lejeune 1985; Wernerus 1988; Wernerus et al. 1989).

Smaller males have mature testes and sperm that are capable of fertilizing eggs (Warner and Lejeune 1985). They move freely between nests (Lejeune 1985; Taborsky et al. 1987) and do not provide any care or defense of the eggs (Taborsky et al. 1987). These males adopt one of two behaviors. The smallest males, termed "sneakers," spawn parasitically and move between nests in response to changes in spawning rates. Intermediate males, called "satellites," parasitize spawns but also chase sneakers away from the nest and are tolerated by the nesting males (Voss 1976; Taborsky et al. 1987).

Multiple studies on female choice in this species have failed to show a relationship between mating success and

any physical male or nest characteristic, even though nesting male success varies greatly (Wernerus 1985, 1988; Wernerus et al. 1987, 1989; van den Berghe et al. 1989). Females appear to spawn with a greater frequency in nests that already contain eggs (Wernerus 1988) and prefer nests without sneaker males (van den Berghe et al. 1989). It has been argued that females of this species do not choose males but instead are choosing spawning situations (Wernerus 1988; Wernerus et al. 1989).

On average, nesting males experience sperm competition 23% of the time (Warner and Lejeune 1985). However, individual nesting males may experience everything from no sperm competition to as many as 20 males surrounding their nest and all of their mating occurring under sperm competition. Despite this variation, the risk of sperm competition is predictable. The probability of a spawn being sneaked increases with the number of sneakers present, and the sneakers hover in full view of the nest (Lejeune 1985; Wernerus et al. 1987). In contrast to nesting males, sneakers and satellites always experience sperm competition, though the intensity of competition is higher for sneaker males (Taborsky et al. 1987). We would expect sneaker, satellite, and nesting males to differ in the number of sperm released per mating. Sneakers have higher relative gonad weights than nesting males, and satellite males have intermediate relative testes size (Warner and Lejeune 1985). Males do not switch alternative behaviors within a reproductive season (Taborsky et al. 1987). However, analysis of growth data indicates that males may switch behaviors between seasons (Alonzo et al., in press). However, individual males could alter their allocation to guarding versus sperm production within a season as their perceived risk or intensity of sperm competition changes.

The expected fitness benefits of behaviors that may represent alternatives to sperm allocation should also change with male size. Because size often determines the winner of aggressive interactions, the efficacy of mate guarding should increase with the relative size of the male. It is important to remember that successful mate guarding reduces the risk of sperm competition. As a result, male allocation strategies may depend not only on the level of sperm competition but also on an individual's efficacy in mate guarding and the cost of sperm production. In *S. ocellatus*, the smallest males in the population, sneakers, may be incapable of effectively guarding mates, while satellites can exclude sneakers but not nesting males from their mating attempts. Consequently, male alternative reproductive behaviors may differ in their sperm production.

On the basis of past models, we would expect nesting males to produce fewer sperm per spawn than satellite or sneaker males, and individual males should increase their sperm production as the risk of sperm competition increases (Parker 1990a, 1990b). For example, nesting males

might alter their sperm allocation depending on their risk of sperm competition. We first examine a general model that predicts the circumstances under which males should allocate energy to mate guarding rather than additional sperm production. We then compare predictions of the model to the allocation pattern of nesting males and examine differences between male alternative behaviors in sperm production in *S. ocellatus*. Considering allocation among behaviors that affect sperm competition should increase our ability to explain and to predict sperm allocation patterns.

Allocation to Mate Guarding or Sperm Production: A Model

Previous models have considered the question of sperm allocation and expenditure (e.g., Parker 1982, 1990a, 1990b; Parker et al. 1996, 1997; Shapiro and Giraldeau 1996; Galvani and Johnstone 1998). However, these models have not examined the situation where males may allocate energy between sperm production and other behaviors that influence sperm competition. We consider the situation where individual males may allocate energy either to sperm production or to mate guarding. We assume that males always produce sufficient sperm to fertilize a female's eggs but that males may use available energy toward additional sperm production or mate guarding. We assume that males can predict their sperm competition risk and average intensity but do not know whether a given mating will be in sperm competition. We also assume that males can reallocate energy with changes in their expected risk or intensity of sperm competition.

Using the same basic model, we consider two different situations. First, mate guarding may decrease the risk of sperm competition. For example, nesting males do not always experience sperm competition. In contrast, other males, such as sneakers or satellites, always spawn in the presence of other males. In this second case, males may, however, reduce the intensity of sperm competition by allocating to mate guarding. We make predictions about allocation between mate guarding and sperm production as a function of male alternative reproductive behaviors as well as within a behavior as the risk or intensity of sperm competition increases.

Case 1

First, we focus on the situation where males can decrease the risk of sperm competition through mate guarding. Let p_G represent the risk of sperm competition when guarding and p_S the risk when allocating to sperm production. Allocation to mate guarding decreases the probability that a given spawn will be joined by another male

so $p_G < p_S$. Let S_G represent the amount of sperm produced per mating by a male allocating energy to mate guarding and S_S represent the amount of sperm produced per mating on average by a male allocating energy to sperm production where $S_G < S_S$. We assume that sperm competition takes the form of a fair raffle, where the proportion of total sperm contributed by a male determines expected paternity (Parker 1982). Let I represent the amount of sperm contributed by other males or the intensity of sperm competition. For simplicity, we assume that I is independent of the allocation pattern adopted. However, if mate guarding also decreases the intensity of sperm competition (e.g., $I_{\text{guard}} < I_{\text{sperm}}$), this is equivalent to a smaller difference existing between S_S and S_G and the basic predictions remain the same.

The expected reproductive value of allocating to sperm production V_S is then

$$V_S = p_S \left(\frac{S_S}{S_S + I} \right) + (1 - p_S). \quad (1)$$

The reproductive value of allocating energy toward mate guarding V_G is

$$V_G = p_G \left(\frac{S_G}{S_G + I} \right) + (1 - p_G). \quad (2)$$

For males to allocate energy to mate guarding over sperm production, the value of guarding must exceed the value of additional sperm production:

$$V_G > V_S, \quad (3)$$

which implies that

$$\frac{(S_S/S_G) + (I/S_G)}{1 + (I/S_G)} = \frac{(S_S + I)}{(S_G + I)} < \frac{p_S}{p_G}. \quad (4)$$

In other words, males are predicted to allocate available energy to mate guarding when the effectiveness of mate guarding in reducing the risk of sperm competition (p_S/p_G) is greater than the effectiveness of sperm production in achieving fertilizations ($(S_S + I)/(S_G + I)$). We can see that the effectiveness of sperm production will decrease as the intensity of sperm competition (I) increases. In this model, the absolute amount of energy available will not affect the behavior adopted. Assume S_S is proportional to S_G and I . We can then predict, given the effectiveness of mate guarding (p_S/p_G), and the relative amounts of sperm produced (S_S/S_G), whether males are predicted to allocate energy to mate guarding or sperm production (fig. 1). As the intensity of sperm competition increases, mate guard-

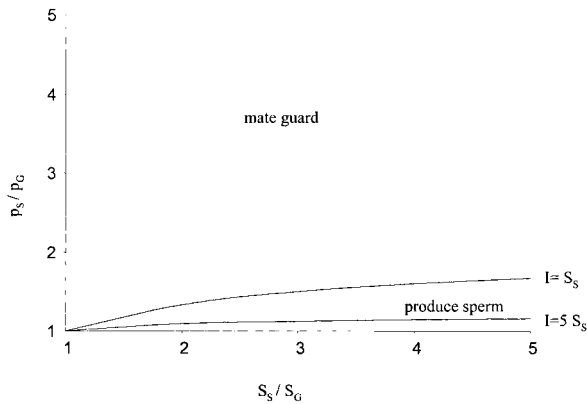


Figure 1: Mate guarding often leads to higher expected reproductive success relative to that achieved through allocation to sperm production. On the X-axis is the relative amount of sperm produced by a male allocating to sperm production versus mate guarding (S_s/S_G) while on the Y-axis is the relative effectiveness of allocating to mate guarding in reducing the risk of sperm competition (p_s/p_G). Given these parameter values, we can predict for a relative level of sperm intensity whether males will have higher reproductive success from allocating energy to sperm production or mate guarding. Each line depicts the boundary between which males are predicted to mate guard (above the line) or allocate to sperm production (below the line) for two different sperm competition intensities.

ing is the preferred behavior for an increasing proportion of the parameter space. Even in the low-intensity situation ($I = S_s$), males are predicted to have higher expected reproductive success if they allocate to mate guarding for most of the parameter space (fig. 1). Even if the intensity of competition is very low ($I \ll S_s$), males are predicted to allocate to mate guarding as long as $S_s/S_G < p_s/p_G$. In most cases, mate guarding is preferred over allocation to additional sperm production. We assumed for simplicity that mate guarding did not reduce the intensity of sperm competition. However, if guarding decreased the risk as well as the intensity of competition, this would favor mate guarding, and males would be predicted to mate guard over an even larger amount of the parameter space.

Both the effectiveness of mate guarding (p_s/p_G) and relative sperm production (S_s/S_G) will affect whether males are predicted to guard or produce sperm. If the effectiveness of mate guarding increases with male size, small males (having small p_s/p_G) may allocate to sperm production while larger males allocate to mate guarding. Similarly, if the relative difference in the amount of sperm produced decreases with size, large males may allocate to mate guarding while smaller males do not. If either p_s/p_G or S_s/S_G is affected by male size, a size-dependent allocation pattern may be predicted, where males allocate to sperm production when small and mate guard when large. With in-

creased intensity of sperm competition, the size at which males should allocate to guarding will decrease.

Alonzo and Warner (1999) showed that, in *Symphodus ocellatus*, nesting males can reduce their risk of sperm competition by 50% when involved in mate guarding. If $p_s/p_G = 2$, we would predict that males should allocate any additional energy to mate guarding rather than sperm production independent of the absolute risk or intensity of sperm competition (fig. 1). In *S. ocellatus*, the amount of sperm released per spawn by nesting males is not expected to increase with increased risk of sperm competition. Instead, we would expect males to allocate any available energy to mate guarding.

Case 2

In the previous model, we focused on males that could avoid sperm competition by guarding females. However, even when males are not able to exclude other males, they may invest in mate guarding to decrease the intensity of sperm competition. In *S. ocellatus*, both sneakers and satellite males always experience some sperm competition from nesting males (p_G and $p_s = 1$). However they might invest energy in mate guarding to decrease the intensity of sperm competition (number of other sneakers or satellites joining them). Let I_s represent the intensity of sperm competition from other parasitic males when a satellite or sneaker male allocates toward sperm production and I_G , if they allocate toward mate guarding. Let S_N represent the amount of sperm produced by the nesting male. Then $I = I_s + S_N$ for satellite or sneaker males allocating to sperm production and $I = I_G + S_N$ for males allocating to mate guarding. Substituting $p_G = 1$, $p_s = 1$, and $I = I_s + S_N$ into equation (1) gives us the expected reproductive value of allocating to sperm production:

$$V_s = \frac{S_s}{S_s + I_s + S_N}. \quad (5)$$

Similarly, the expected reproductive value of allocating energy toward mate guarding will be

$$V_G = \frac{S_G}{S_G + I_G + S_N}. \quad (6)$$

For males to allocate energy to mate guarding over sperm production, the value of guarding must exceed the value of additional sperm production, which implies

$$\frac{S_s}{S_G} < \frac{I_s}{I_G}. \quad (7)$$

Therefore, when males are always in sperm competition

but can decrease the intensity of sperm competition by mate guarding, they should guard when the effectiveness of mate guarding in reducing the intensity of sperm competition (I_s/I_G) is greater than the effectiveness of allocating to sperm production in increasing fertilization (S_s/S_G). If I_s/I_G increases with size or S_s/S_G decreases with size, larger males should allocate to guarding while smaller males allocate to sperm production.

In *S. ocellatus*, sneaker males tend to be the smallest males in the population and may be unable to effectively guard mates ($I_s/I_G \approx 1$). Satellite males tend to be the largest nonterritorial male at each nest and often are only in sperm competition with the nesting male (Taborsky et al. 1987). Thus, they appear to be able to guard effectively against sneakers (I_s/I_G large). As a result, sneakers are predicted to allocate to sperm production and satellites to mate guarding. We would not expect satellites to alter their sperm production with increased intensity or risk of sperm competition, while sneaker males might change their ejaculate size with increased sperm competition.

In summary, we predict that, in *S. ocellatus*, small sneaker males should produce more sperm per spawn than nesting males. Further, satellites and nesting males (who can be successful in mate guarding) should not increase their sperm production with increased risk of sperm competition. Satellite males are expected to produce fewer sperm per spawn than sneaker males because they allocate energy toward mate guarding to decrease their intensity of sperm competition. We examine these predictions below by estimating the amount of sperm released in pair spawns, spawns joined by satellite males, and spawns joined by sneaker males. We predict that spawns with sneaker males should have significantly more sperm than pair spawns or spawns sneaked by satellite males. Furthermore, we examine nesting male sperm production as the risk of sperm competition varies between nesting males. Our model predicts that nesting males should not increase their sperm expenditure with increased risk of sperm competition.

Methods

Study Site and Species

Symphodus ocellatus is a Mediterranean wrasse found on rocky and seagrass substrates in shallow coastal waters (Fiedler 1964). Reproductive behavior is easily observed along the coast of Corsica (Fiedler 1964; Voss 1976). During the breeding season (May–July), mating occurs daily from sunrise to sunset (Lejeune 1985). Individuals live up to 3 yr (Warner and Lejeune 1985) and can be marked using subcutaneous injections of alcian blue (Lejeune 1985). There is no evidence for sex change in this species (Warner and Lejeune 1985; Bentivegna and Benedetto

1989). All research was conducted between May and July 1994 under natural conditions near the University of Liège (Belgium) Marine Laboratory, La Station de Recherches Sous-Marin et Océanographique (STARESO), located near Calvi, Corsica, France. *Symphodus ocellatus* is found at high densities in Revellata Bay near the research station. All observations were made within 200 m of shore above 15-m depth using SCUBA. Nesting males were caught at the beginning of the reproductive season and given individual marks using a subcutaneous injection of alcian blue. These marks could be used to recognize individuals throughout the season. The research area was surveyed daily to identify the existence of new nests and the spawning activity at existing nests.

Behavioral Observations

Before sperm collection, nests were observed for 10 min to determine the mating rate, number of parasitic males present at the nest, number of chases directed toward parasitic males by the nesting male, and the proportion of spawns sneaked at the nest. The observer remained at least 3 m from the nest. Every minute, we noted the number of parasitic males (sneakers + satellites) within 3 m of the nest and took the average number of parasitic males present at the nest over the 10-min period. Distances were estimated visually. Females, sneakers, and nesting males can be differentiated by their behavior and morphology. Males do not switch behavior (nesting males, sneakers, or satellites) within a reproductive season. Nesting males have a distinct color pattern and are larger than all other individuals in the population, which easily distinguishes them from parasitic males and females. Satellites can be distinguished from sneakers by the satellite male's submissive behavior toward the nesting male and aggression toward sneaker males (Taborsky et al. 1987). Females can be distinguished from parasitic males by the absence of a brightly colored spot on their opercula, the shape of their abdomen and genital papillae, and the absence of aggressive behavior by the nesting males toward them. We counted the number of females spawning in the nest. For each female, we recorded the number of times she spawned and the number of spawns joined by sneakers. We collected 149 sperm samples with observations at 49 nests.

Sperm Collection

Before sperm collection, any sperm in the nest from previous spawning events was removed by flushing the nest using a 750-mL syringe. Samples taken directly following the flushing of the nest indicated that no sperm was present postcleaning ($n = 4$). The observer then waited for the next spawn to occur and noted the number and type of males

involved in the spawn. Immediately following a spawning event, the tip of a large (750 mL) syringe was placed in the nest, and water was collected until the syringe was full. The sample was taken immediately to the surface and placed in a collection bottle with 10 drops of rose bengal. After 20 min, 10 mL of formalin were added to preserve the sperm. Postcollection samples were also taken to determine whether sperm remained in the nest following the sperm collection ($n = 18$).

Sperm Number Estimation

We use a version of a method developed by Shapiro et al. (1994) to estimate sperm number released by pelagically spawning fish. First, the sample was gravity filtered through a 30- μm mesh to remove small debris from the sample, and the total volume of the sample was taken. A 20-mL subsample was vacuum filtered onto a millipore filter (0.22- μm pore size). This collected the sperm onto the surface of the millipore filter. The filter paper was then dried, mounted on a slide, and cleared with immersion oil. Filters were viewed under a light microscope at $\times 400$ magnification. Using an ocular grid (0.295 mm² in total area), we counted the number of sperm appearing in the grid for 20 randomly selected positions on the filter. Total number of sperm was estimated using the known area of the grid, filter area, subsample volume, and total sample volume. Sperm estimation was done blind with respect to the type of sample and situation at the nest. In two cases, we filtered five separate filters from the same sample to verify the consistency of the estimation method. We also had the two individuals involved in estimation count the number of sperm in the same grid to control for inter-observer bias ($n = 4$). Both observers consistently counted the same number of sperm per grid.

Collected Spawns

For males with multiple samples, we randomly selected one sample from each male to create a data set of 49 independent samples. We also collected nine spawns that involved a nesting male and a satellite male and 14 spawns involving one nesting male and one sneaker male. To examine the possibility of sperm depletion, we collected a pair spawn from 15 males both in the morning and in the late afternoon. To examine within-male variation, we also collected two pair spawn samples from a nesting male immediately following one another ($n = 16$), and 2 d in a row ($n = 14$).

Statistical Analyses

We first made comparisons between nesting, satellite, and sneaker male production by comparing the sperm per spawn in situations with only the nesting male, nesting male and a satellite, and the nesting male and a sneaker male. Because sperm per spawn deviated significantly from normality in these samples according to a Shapiro-Wilk's test (Shapiro and Wilk 1965; Zar 1996), sperm per spawn was power transformed. A one-way ANOVA with Tukey pairwise comparisons was then performed on the transformed data.

We also calculated the Pearson correlation coefficient between the number of parasitic males present at the nest on average during an observation and the proportion of spawns sneaked to verify the positive relationship between sneaker and satellite male presence and risk of sperm competition. We then calculated the Pearson correlation coefficient between the number of parasitic males present at the nest and the number of nesting male chases, between the number of parasitic males and the sperm produced by the nesting male in pair spawn, and the mating rate at the nest and the sperm produced by the nesting male. For these calculations, we used the set of 49 pair spawns from 49 different males. We tested each correlation calculated for significant differences from 0. For any correlation not significantly different from 0, we also calculated the power of the test (Zar 1996). To examine sperm depletion, we compared morning and afternoon samples using a Wilcoxon Sign-Rank test. We collected three sets of samples to examine within-male variation in sperm production. For each set of samples, we calculated the Spearman rank correlation coefficient and tested whether the rank correlation was significantly different from 0.

Results

Controls

All postcleaning samples were found to have zero sperm, indicating that cleaning successfully flushed any preexisting sperm from the nest. Sperm was found in postcollection samples. Thus, the collection method did not collect all of the sperm released. However, the amount of sperm remaining in the nest was small relative to the initial collections ($\bar{X} = 0.27 \times 10^6$, $n = 18$ in postcollection vs. 1.3×10^6 , $n = 49$ in initial collections of pair spawns, and 5.4×10^6 , $n = 14$ in sneaked spawns). Furthermore, samples collected from the same male in close succession are very concordant ($r_s = 0.75$, $P = .002$). Although the sperm remaining after collection decreases the accuracy of our estimates of sperm number actually released, the sperm initially collected is a good proxy for the initial concentration of sperm released. If anything, the estimated sperm

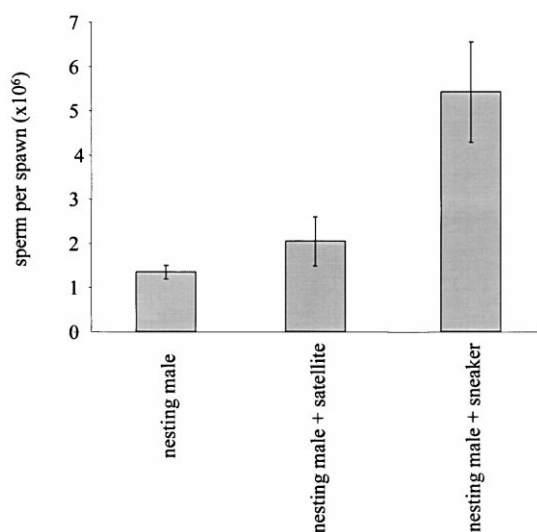


Figure 2: Alternative reproductive behaviors differ in the number of sperm released per mating. Means \pm SE are shown. If we assume that additional sperm is not contributed by the nesting male, sneakers produce significantly more sperm than either nesting males or nesting males and a satellite male. Nesting males experience low-intensity sperm competition from satellites but much more intense sperm competition from a single sneaker.

numbers will be slight underestimates of the total sperm released. Comparison of multiple filters for the same samples showed the estimation technique to be very consistent.

Comparison between Male Alternative Reproductive Behaviors

We collected pair spawns ($n = 49$), spawns that included a nesting male and a satellite male ($n = 9$), and spawns that included a nesting male joined by one sneaker male ($n = 14$). Sperm per spawn deviated significantly from normality according to a Shapiro-Wilks test ($W = 0.54$, $P < .001$). We therefore power transformed the data (sperm^{0.15}) to conform to the assumption of normality ($W = 0.97$, $P = .31$). We found significant differences between the estimated sperm per spawn released between these three situations ($F = 16.70$, $P = .0001$). Spawns including a nesting male and a sneaker male had significantly more sperm than pair spawns or spawns with satellite males (fig. 2).

Nesting Male Sperm Allocation and Within-Male Variation

Nesting males were consistent in their sperm production in the short term. A significant positive relationship existed between sperm samples taken from spawns immediately following one another ($r_s = 0.75$, $P = .002$), while samples

taken further apart in time from the same male were not significantly correlated (morning/afternoon $r_s = -0.22$, $P > .20$; next day $r_s = -0.03$, $P > .50$). Sperm released by a nesting male in the morning was not significantly different than sperm per pair spawn released in the afternoon ($T = 43$, $P > .50$).

A significant positive correlation was found between the mean number of parasitic males present at the nest and the proportion of spawns sneaked ($r = 0.42$, $P = .007$). However, no significant correlation existed between sperm production per spawn and number of parasitic males ($r = -0.03$, $P = .84$, $1 - \beta = 0.97$; fig. 3) or sperm per pair spawn and mating rate ($r = 0.06$, $P = .70$, $1 - \beta = 0.97$). There was a positive trend of increased number of chases per unit time with number of parasitic males present ($r = 0.25$, $P = .08$).

Discussion

For nesting males in *Symphodus ocellatus*, a sneaker male represents a high intensity of sperm competition while satellites represent a lower intensity of competition (fig. 2). Nesting males produce 1.3×10^6 sperm per pair spawn on average, and this amount does not change as the risk of sperm competition (represented by the number of parasitic males present) at the nest changes (fig. 3). This occurs despite the fact that sperm competition risk can vary from 0 to 1 over the course of a single nest cycle and the number of parasitic males at the nest clearly predicts the risk of sperm competition. In contrast, nesting males do increase the number of chases they direct toward parasitic males as the number of parasitic males at the nest (and

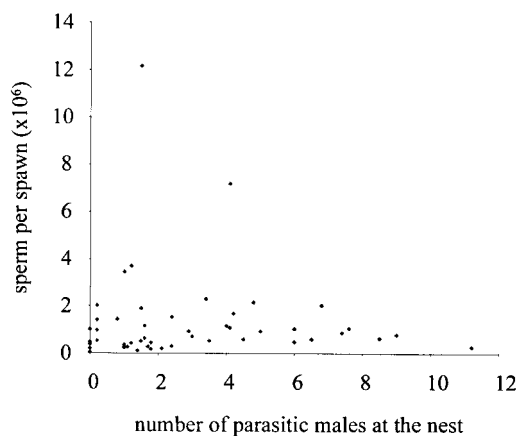


Figure 3: Sperm released per pair spawn does not increase with the mean number of parasitic males present at the nest during a 10-min observation before sperm collection. Males do not appear to alter their pattern of sperm allocation with changes in the risk of sperm competition.

thus the risk of sperm competition) increases. Thus nesting males increase their time spent mate guarding as the risk of sperm competition increases.

As expected, sneakers contribute significantly more sperm than nesting males, while satellite males do not appear to produce significantly more sperm per spawn than nesting males. Similar to nesting males, satellite males may be allocating to mate guarding rather than additional sperm production. However, satellite males decrease only the number of sneaker males joining their spawn rather than reducing the risk of sperm competition. Small sneaker males may be unable to guard against larger satellite and nesting males and thus allocate to sperm production instead of mate guarding.

In other fish species, males allocate sperm between matings based on female fecundity, expected mating rate, and local environmental conditions (Shapiro et al 1994; Marconato et al. 1995; Warner et al. 1995; Marconato and Shapiro 1996; Warner 1997; Rasotto and Shapiro 1998). Thus the apparent absence of allocation by nesting males in *S. ocellatus* is striking, especially given the extreme and predictable variation in sperm competition. Models that focus only on sperm expenditure predict larger expenditures with increased risk of sperm competition. Differences between male tactics within a species and interspecific variation show increased sperm production with increased risk of competition (e.g., Warner and Lejeune 1985; Gage 1994; Gage et al. 1995; Stockley et al. 1997). As observed in many other species, we found that sneakers produced more sperm per spawn than nesting males. However, satellite males did not produce more sperm than nesting males. This pattern is consistent with the idea that satellite males allocate energy to mate guarding rather than sperm production. Past theory predicts that sperm production should also increase with risk within a tactic, although this pattern has not been documented within a species. In contrast, as presented here, a model that allows an alternative allocation to mate guarding predicts that males will respond to risk by increasing guarding rather than sperm production. Our results are consistent with the predictions of this model.

While the increase in aggression toward parasitic males with increased sneaker presence and risk of sperm competition is consistent with the pattern of allocation to mate guarding rather than sperm production, other possible explanations exist for the lack of allocation, as follows.

Precise Responses to Specific Spawns

We have assumed that nesting males do not produce different amounts of sperm when in a pair spawn or sneaked mating and can only respond to an overall increase in risk with a general increase in sperm output per spawn. How-

ever, if males can precisely predict whether a particular mating will occur in sperm competition, then this could explain the lack of allocation seen among pair spawns. However, it is unlikely that nesting males of this species adjust the amount of sperm released between sneaked and pair spawns. Sneakers enter the nest shortly after the female and nesting male initiate spawning, and thus it is most realistic to assume that the additional sperm seen in such spawns is contributed mainly by these peripheral males. Nesting males produce smaller total amounts of sperm than sneaker males (S. H. Alonzo, unpublished data), and our interpretation is consistent with the observed differences in relative gonad weights (Warner and Lejeune 1985). Spawns consisting of nesting males joined by satellite males show only marginally higher sperm numbers than pair spawns, while spawns joined by sneakers have almost five times as much sperm. It seems reasonable to assume that this difference is due to sperm production by satellites versus sneakers rather than additional sperm release by nesting males only in the presence of sneakers. Males could be simply unable to alter the amount of sperm released after a spawn has been initiated. While in other wrasses males show an impressive ability to control the amount of sperm released per mating, these responses are to cues available before a spawn begins (e.g., Warner et al. 1995; Rasotto and Shapiro 1998).

Sperm Limitation

The risk of sperm competition and mating rates positively co-vary. Males could be sperm limited at high mating rates and have abundant sperm at low mating rates, and this could act against any tendency to increase sperm release under risk of competition. However, no pattern was observed between sperm release and mating rate at the nest. Similarly, no evidence for sperm depletion over the course of the day was found.

Precision of Data

Finally, it is also possible that our samples are too variable to detect a subtle pattern of allocation. However, two samples collected in close succession from the same male were extremely concordant and indicate that our sampling can detect a consistent concentration of sperm released. Variation in sperm production among males could potentially have major effects on fitness and warrants further study.

In conclusion, nesting males do not increase the amount of sperm they release per mating as the risk of sperm competition increases, and models that focus only on sperm expenditure cannot explain this pattern. In contrast, a model allowing energy allocation to mate guarding predicts the observed pattern of sperm allocation. Similarly,

by considering allocation to mate guarding, we can explain the difference between sneakers and satellites in sperm production. In general, the model predicts that mate guarding by males with a high likelihood of winning contests will usually lead to higher expected reproductive success than additional sperm production. A focus on sperm expenditure alone can miss part of the picture. This will especially be the case when males differ in their roles and their ability to mate guard or can otherwise affect their own risk and intensity of sperm competition. Predictive theories of sperm allocation need to consider allocation among alternative behaviors that can directly affect sperm competition as well as the temporal dynamics of energy allocation among matings. Multiplayer, state-dependent game models (Alonzo and Warner 2000) are presently being developed to examine the energy allocation between mate guarding and sperm production as a function of male size and life history. In order to make realistic quantitative predictions, there is also a need to study the relationship between behaviors such as mate guarding and their effect on sperm competition. The effectiveness of mate guarding and the trade-off between sperm production and guarding can drive variation in sperm allocation within and between species. In order to have a theory of sperm competition that will allow us consistently to predict and to explain patterns of sperm allocation, we need to broaden our view of sperm competition to include more than just sperm.

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