Sexual conflict: males with highest mating success convey the lowest fertilization benefits to females

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SUMMARY

In natural populations of a coral reef fish (the bluehead wrasse, Thalassoma bifasciatum), males with the highest daily mating success produce the fewest sperm per mating, and this is reflected in significantly lower fertilization rates. The average amount released by males in pair-mating was $3.3 \times 10^8$ spermatozoa, resulting in a fertilization rate of 96%. Sperm released per spawn declined with increasing mating success, so that females mating with the most successful males had less than 93% of their eggs fertilized. It is unlikely that high mating-success males are physiologically incapable of increasing sperm production, because younger males with different mating strategies have absolutely larger testes and higher daily sperm output. Feeding experiments suggest that high-success males are diverting energy from gamete production to other fitness-enhancing activities such as mate guarding. Females incur the cost of low sperm release by having fewer of their eggs fertilized. There are no obvious compensatory benefits to females from mating with high-success males.

1. INTRODUCTION

Whereas both males and females have a fitness interest in the successful completion of mating, the tactics involved in achieving that success may differ substantially between the sexes (Trivers 1972). Sexual conflicts over the provisioning of young or the timing and location of mating have been extensively discussed (for examples, see Davies 1989; Slagsvold & Lifjeld 1989; Simmons & Gwynne 1991; Hebert & Sealy 1993), but there has been little consideration of the effects of gamete allocation strategies of one sex on the fitness of the other. The reason for this is that only recently have we realised that males do not always have a superabundance of sperm (reviewed in Pitnick & Markow 1994), and that the supply of male gametes may strongly limit the proportion of a female’s eggs that can be fertilized (for examples, see Levitan et al. 1992; Dudash 1993; Levitan & Petersen 1995). If sperm are costly to produce, and if energy could more profitably be expended on other fitness-enhancing activities, increased mating demands may lead a male to reduce sperm output per mating (Parker 1990a, b; Shapiro & Giraldeau 1995). Partitioning of sperm among matings may lead to reduced fertilization within each mating, but it is less costly to the male than becoming sperm depleted before mating opportunities have ceased. While highly adaptive for the male, sperm partitioning can incur fertilization costs to the female. Here, we investigate how males of the bluehead wrasse (Thalassoma bifasciatum) partition their sperm among multiple matings in a day, and how such partitioning affects fertilization rate. We further investigate male allocation decisions by providing supplementary food to males and tracking subsequent changes in sperm production and mating success.

2. METHODS

We monitored the daily mating success of 33 territorial males on patch reefs in Tague Bay, St. Croix, U.S. Virgin Islands (17°45’N, 64°35’W). On Tague Bay patch reefs, most females feed on plankton on the upcurrent ends of reefs during the course of the day, and migrate to specific downcurrent mating sites for a single brief visit (Warner & Schulte 1992). Individual Terminal Phase (TP) colouration males each occupy a spawning site (Warner 1988; Hensley et al. 1994), and defend the site against smaller, younger males in the Initial Phase (IP) colouration (Warner & Hoffman 1980; Warner 1984). TP males individually add their sperm to the matings of TP males by rushing in at the apex of the spawning ascent, or they may arrive en masse, usurp the mating site, and mate in groups with arriving females. Mating takes place daily throughout the year. Fertilization is external, the eggs are pelagic, and there is no parental care. On average, females spawn two out of every three days, and tend to be faithful to particular mating sites (Warner 1984). This fidelity leads to consistent differences between TP male sites in average daily spawning rates, from less than five to more than 80 matings per day (mean $\pm$ s.d. = 35.7 $\pm$ 21.0; $n = 33$ males, 5 days per male).

To analyse sperm output and fertilization rate, we followed the protocols given in Shapiro et al. (1994). Gametes were
captured in large plastic bags of known volumes by divers within three seconds of spawning. Sperm were subsampled, stained, filtered, and counted at 400× magnification. The total number of sperm released in the spawn was estimated by multiplying the number of counted sperm by a dilution factor appropriate to the volume of the collecting bag. A subsample of eggs was stained and scored for fertilization 2 h after spawning, which allows ample time for development. We did not use any sample in which fewer than 20 eggs were present in the subsample. The number of eggs released by a female in each spawning varies greatly, depending on the size of the female and the amount of energy devoted to egg production on any particular day (Schultz & Warner 1990).

To investigate energy allocation in \( \text{TP} \) males, we performed three food augmentation experiments on separate reefs in June–September, 1994. Five experimental and five control males were observed during a two-week premanipulation period. The experimental males were then fed ad lib once a day (after spawning) on small pieces of conch flesh for two weeks. Experimental data began to be taken three days after the initiation of feeding, and continued for two weeks. During the pre-feeding and experimental periods, observers recorded the total number of spawns occurring at the male’s site per day, the number of spawns lost to intruding \( \text{TP} \) males, and the sperm output for sampled spawns of each male. Mean sample size was 6.6 observation days and 28.2 sperm samples per male for the control and experimental periods. Mean daily mating rates ranged between 17.5 and 83.0 matings per day at the experimental sites, and between 26.0 and 56.8 matings per day at control sites. For each male studied, there were no significant differences between the premanipulation and experimental periods in the total number of spawns occurring per day at the mating site.

3. RESULTS AND DISCUSSION

(a) Fertilization and sperm allocation as a function of mating rate

The proportion of eggs fertilized in any one mating was significantly related to the amount of sperm released by the male, but was independent of the number of eggs contributed by the female (see Table 1). Fertilization rates initially increased rapidly with sperm numbers; at about 25 million sperm released, 95% of the eggs are fertilized. As sperm numbers increased, the effect on fertilization rate declined (see figure 1). The average amount of sperm released by \( \text{TP} \) males in pair matings was \( 3.33 \times 10^8 \) (s.e. = 0.07 × 10⁸; \( n = 1669 \) matings). In contrast, smaller \( \text{TP} \) males, which nearly always spawn under conditions of sperm competition, have much larger testes (0.12 g versus 0.04 g; Warner & Robertson 1978) and higher individual sperm output per spawn (mean ± 1 s.e. = 52.8 ± 6.9 × 10⁶; \( n = 27 \); Shapiro et al. 1994). This pattern of sperm output per mating is expected when males differ greatly in the amount of sperm competition that they chronically face (Parker 1990b). At this point, we are unable to determine if \( \text{TP} \) males can temporarily increase their sperm output per spawn when faced with sperm competition.

Males with high average daily mating rates released significantly fewer sperm per spawn than did males with lower mating rates (figure 2). As expected from

![Figure 1. Proportion of eggs fertilized as a function of the amount of sperm contributed by a male. Data are based on 1669 pair matings collected May–September in 1993 and 1994. Shown are the mean (±95% confidence interval) values for proportion fertilized versus the number of sperm contributed for each of seven arbitrary sperm-count categories. Categories were, in millions, with \( n \) in parentheses: 0–0.25 (12), 0.26–0.50 (52), 0.51–0.75 (80), 0.76–1.00 (111), 1.01–2.00 (423), 2.01–5.00 (680), and >5.01 (311). The curve (calculated from the raw data) is a Michaelis–Menten relation fitted by the Marquardt–Levenberg algorithm (Press 1992), where proportion eggs fertilized = \( (0.994 \times \text{sperm})/(0.117 + \text{sperm}) \), sperm expressed in millions.](image1)

![Figure 2. Sperm output per spawn and measured fertilization rates obtained by males with different average daily mating rates. Shown are mean sperm output per spawn (empty square) and mean proportion of eggs fertilized (filled triangle) versus mean daily mating rates for 33 different \( \text{TP} \) males. Mean of 26.97 matings per male were collected. Correlations: mean sperm per spawn versus mean daily mating success, \( r = −0.583, p < 0.0001 \); arcsin square root of mean proportion of eggs fertilized versus mean daily mating success, \( r = −0.718, p < 0.0001 \).](image2)

<table>
<thead>
<tr>
<th>Eggs released &amp; number of eggs released on the proportion of eggs fertilized</th>
<th>( \beta )</th>
<th>( \tau )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>eggs</td>
<td>0.054</td>
<td>1.54</td>
<td>0.125</td>
</tr>
<tr>
<td>sperm</td>
<td>0.311</td>
<td>6.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>interaction</td>
<td>−0.087</td>
<td>−1.46</td>
<td>0.146</td>
</tr>
</tbody>
</table>

Table 1. Effects of amount of sperm released and number of eggs released on the proportion of eggs fertilized

(Analysis based on 1669 spawns. Overall adjusted \( r^2 = 0.062 \). Shown are the standardized partial regression coefficients, \( \beta \).)
the fertilization curve (figure 1), this difference resulted in lower average fertilization rates for the high mating-rate males (figure 2). Males mating near the average rate (between 20 and 49 matings per day) fertilized a mean of 96.0%, of their mate’s eggs, while males with more than 50 matings per day averaged 92.7%. Males with ten or fewer spawners per day released large amounts of sperm per spawn and provided the highest fertilization rate to their females (98.3%).

Total daily sperm output can be estimated by multiplying mean daily mating rate by the average sperm released per spawn. For all Tp males, mean sperm output was $119 \times 10^8$ sperm per day. Males mating 90–100 times per day produced, on average, 4.3 times as much sperm each day ($180 \times 10^8$ sperm) as did males spawning 4–10 times per day ($42 \times 10^8$ sperm). If the highest-success males had not reduced the amount of sperm released per spawn, their total sperm output would be much higher (over $315 \times 10^8$ sperm per day, assuming the average release of $3.33 \times 10^8$ sperm per spawn).

(b) Male allocation strategies

In other species, low sperm release is clearly the result of depletion of sperm reserves subsequent to more successful matings (for examples, see Nakatsuru & Kramer 1982; Birkhead & Fleicher 1995), which can occur when males are provided with an unexpectedly high number of mates. However, the low average number of sperm released in each mating for the highest-success males of T. bifasciatum (mean of 1.9 million sperm per spawn for the two males with the highest spawning rates) was not caused by sperm depletion over the course of the mating period. Tp males have predictable daily mating success (Warner 1987) and appear to partition their sperm production such that they do not deplete in later matings. Both sperm output per spawn and fertilization rates remained low over the entire mating period, with no downward trends (Petersen et al. 1992; Shapiro et al. 1994). Given the shape of the fertilization curve (figure 1), reduction of sperm released in all spawns yields a higher number of eggs fertilized than a strategy of releasing higher levels of sperm in early spawns and depleting towards the end of the spawning period (Pitnick & Markow 1994; Shapiro & Giraldeau 1995). While adaptive for the male, sperm partitioning negatively affects female reproductive success uniformly across the mating period.

Tp males in the bluehead wrasse release more sperm when mating with larger females (Shapiro et al. 1994), but there was no change in the size structure of mating females across territories with different daily mating rates (unpublished data). Thus, changes in female size structure could not account for the drop in fertilization rate that accompanied increases in daily mating success.

It is commonly assumed that in the absence of sperm competition, the number of sperm released should be at least sufficient to ensure fertilization (for examples, see Parker 1990a). These results appear contrary to these expectations. Why do successful males not release more sperm? Tp males certainly appear physiologically capable of producing higher quantities of sperm per day. Large Ip males become Tp males under the proper social circumstances, and at that time they shift from multiple-male spawning (with chronic sperm competition) to pair-spawning. In parallel with this change, their testis becomes dramatically reduced in size and total sperm output drops (Warner & Robertson 1978; Shapiro et al. 1994). If successful males could produce more total sperm (and thereby increase the amount of sperm contributed to each mating), why do they incur instead a 3–5% drop in fertilization rate (figure 2)? Recent theory contrasting sperm competition with mate guarding suggests that males may allocate energy away from sperm production and toward defense of females when this yields higher rewards in terms of fitness (Parker 1990a,b). We explored this idea with the feeding experiment described below.

We assume, as did Parker (1990a,b), that there is a trade-off between effort spent on sperm production and effort spent on obtaining matings. This trade-off should become apparent when energy supplies are limited, which is more likely to be the case when mating rates are high. Mating site defense by Tp males prevents loss of matings to Ip males; such losses can be more costly than a simple decline in fertilization rate. During the course of our observations, Tp males lost an average of 11.5% of the spawns occurring at their mating sites to Ip males, either completely (when a group of Ip males temporarily took over the site) or partially (when one or more Ip males rushed in to join the Tp male at the moment of gamete release). Assuming the Tp male loses most of his paternity when an Ip male interferes with a spawn, mate guarding may be more profitable than maintaining high sperm output. Consider a Tp male spawning at a site where 50 females arrive each a day, and losing six of them to Ip males: successful defense of a single additional female raises his paternity of available eggs that day by about 2%. Given the relation between fertilization rate and sperm output (figure 1), the same male would have to increase sperm release by over 50% in each spawn in order to accrue a similar 2% increase in success through fertilization. Thus energy devoted to mate guarding may yield higher fitness rewards than the same energy allocated to increased sperm production.

When provided with supplemental food, Tp males did not increase their sperm output per spawn relative to controls (table 2a), nor was there any change in fertilization rates. Sperm released per spawn increased slightly for all males over the course of the experiment, including unfed controls, but there was no apparent effect of food augmentation. Instead, Tp males significantly increased the proportion of arriving females with whom they successfully spawned (table 2b). All males showed increases in mating success from supplemental feeding, and none showed a response in sperm release per spawn. The estimated number of sperm released per day increased for experimental males (because their number of matings increased), but the increase was not reflected in changes in the number of sperm released in each mating. If fitness can
be estimated as the number of matings times the expected gain (see Parker 1990a, b), the average 8% gain in fitness through increases in matings (table 2b) is greater than the 3–5% gain in fertilization rate that high-success (more than 50 matings per day) males could achieve through increased sperm production (figure 2). While males used supplemental food to increase their own fitness, there was no parallel increase in the fitness of their mates, and the sexual conflict remained. Because the fertilization curve drops off sharply at sperm numbers less than a million (figure 1), further reductions in sperm release would lower male fitness much more than 3–5% (see Shapiro & Giraldeau 1995).

Whereas an energy allocation hypothesis conforms well to the sperm release patterns seen in males with high daily mating rates, it does not explain why males with very low mating success release such high numbers of sperm (figure 2), well in excess of that needed for high fertilization rates (figure 1). One possible interpretation of these patterns comes from a consideration of the relative costs of sperm production (Parker 1990a, b). When mating rates are high and energy is limited, the cost of sperm production is relatively high because it can lead to losses in matings; in this case, low sperm numbers per ejaculate are expected. However, at lower mating rates the negative relation between sperm production and effective mate guarding may not apply. In this case, sperm production is less costly and more sperm per spawn can be released as a defence against the probability of a sneak spawn occurring. Previous work has shown that smaller TP males tend to gain lower-success mating sites, and a higher proportion of their spawns occur in sperm competition with sneaking TP males (Warner & Schultz 1992). All else being equal, individuals faced with a higher frequency of sperm competition should release more sperm per mating (Parker 1990b).

(e) Costs to females

It appears that females could increase the proportion of eggs fertilized by mating with a TP male with lower overall mating success. TP males do not coerce or control their mates, and females can freely change mating sites and males (Warner & Schultz 1992); yet females do not appear to avoid reproductively successful males (Warner 1987). In some species, females accrue benefits from mating with particular males, either directly or through improved genetic quality of the young (for example, see Howard 1978; Woodward 1986; Simmons 1987; Andersson 1994). Because the majority of matings on most reefs occur with small TP males at group-spawn sites freely chosen by females, it is not obvious that females gain indirect genetic benefits from mating with highly successful TP males. Equally, the possibilities for direct benefits accruing to the female or her offspring are limited, because there is no parental care given to the pelagic eggs. Mating at high-success sites may offer more safety because of predator dilution, but this effect would be even stronger at crowded group-spawn sites.

At this point, we are unable to identify any clear compensatory benefits that females might gain from mating with high-success males. Females may derive genetic benefits from mating with males with proven survival and reproductive abilities, but the pelagic larval stage prevents us from testing for these benefits. Alternatively, females may simply be unable to estimate the daily mating success of the male during their brief visit to the mating site. Because of this lack of information by females, males that reduce fertilization rates by limiting sperm release may be able to resolve the sexual conflict in their own favour.

4. CONCLUSIONS

Low sperm output appears to be a chronic condition for males in some species where the chances of remating are high (Dewbury 1982; Pitnick & Markow 1994). These males may need to partition their limited sperm supply such that each female receives less than a maximal amount of sperm (Pitnick 1990; Pitnick & Markow 1994). Shapiro & Giraldeau (1995) have suggested that the lower limit of sperm release is set at the point where the ratio of eggs fertilized to sperm released is at a maximum. If females cannot readily remate (Pitnick 1991), or if remating carries costs (Fowler & Partridge 1989), then their fitness may be lowered because of the male strategy. As shown here, in the bluehead wrasse sperm output varies within a species in a manner consistent with predictions based on adaptive sperm partitioning; males with the highest mating demands reduce the amount of sperm contributed to each mating.

Lack of sperm or pollen has been shown to lower fertilization rates in marine invertebrates and plants that depend on long-distance dispersal to commingle.
gametes (Galen 1985; Pennington 1985; Brazeau & Lasker 1992; Levitan et al. 1992; Dudash 1993); here we show that fertilization can also be relatively low under natural circumstances where males and females achieve close contact. Even in the absence of gamete competition, sperm delivery can still limit reproductive success. Sperm production, like other energetically expensive activities, is part of the overall allocation strategy of the male (Parker 1990a, b; Pitnick & Markow 1994). Whereas these allocations may optimize reproductive success for that male, they may simultaneously incur costs to his mates.

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