Male limitation of female reproductive success in a pipefish: effects of body-size differences

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Summary. In the pipefish *Syngnathus typhle*, a species with exclusive male parental care, males limit female reproductive success because of their limited brood pouch space and long pregnancy. Sexual size dimorphism is absent in these 1-year-old animals but increases with age so that older females are larger than similarly aged males. Because fecundity is related to size in both sexes and increases more rapidly with body size in females than in males, the difference in growth increases female fecundity more, relative to male fecundity, as the fish get older. We therefore predicted that male limitation of female reproductive success is even more severe when all age classes are considered. To measure a female's maximum reproductive rate, she was provided with three males. Small 1-year-old females produced as many eggs, or produced eggs at the same rate, as a male of similar size could care for. Small females filled on average 1.06 males within the time span of one male pregnancy and actually produced on average 10 eggs fewer than needed to fill a similarly sized male. Large 2-year-old females, in contrast, produced on average a surplus of 149 eggs and filled 2.7 similarly sized males within the course of one pregnancy. The difference between females of the two size classes was highly significant. Males prefer to mate with larger females if given a choice. In nature sex ratios are equal, and males limit female reproductive success in the whole population. Therefore, small females are more severely constrained by mate availability than are larger females because males choose to mate with larger females.

Introduction

The sex that possesses a combination of behaviors and traits such as strong intrasexual competition for mates, features enhancing such a competitive ability (i.e., weapons), and features increasing mate attractiveness (i.e.,

ornaments) does not limit the reproductive success of the opposite sex, whereas the converse is assumed for the more passive, choosy, and plain sex (e.g., Bateman 1948; Trivers 1972; Williams 1975; Dawkins 1976; Daly and Wilson 1983). The competitive sex role is typically played by males and the choosy role by females, but numerous examples of role reversal exist (e.g., Gwynne 1981, 1984, 1986; Petrie 1983; Thornhill and Alcock 1983; Oring 1986; Fiedler 1954; Breder and Rosen 1966; Berglund et al. 1986a, b, 1988, 1989). Such examples are well suited for testing the relation between sex roles and reproductive limitation. These tests have been done in a few cases only: for instance, male brooding rate may limit female reproductive success in the spotted sandpiper *Actitis macularia* (Maxon and Oring 1980). In the two pipefish species *Nerophis ophidion* (L.) and *Syngnathus typhle* L., males have been shown to limit female reproductive success in 1-year-old fishes by requiring more time for paternal care than females need to produce these eggs (Berglund et al. 1989).

Furthermore, patterns of reproductive limitations may change over the life of an organism, yielding different selective pressures at different times. Patterns of sexual dimorphism are known to change with age, for instance, in many bird species (delayed plumage maturation; e.g., Lyon and Montgomerie 1986) and in sex-changing protogynous fish with primary, female-colored, "sneaky" males (e.g., Warner et al. 1975; Warner and Harlan 1982; Charnov 1982a, b). In both these cases sexual dimorphism increases with age, but it is unknown whether this is associated with changes in the pattern of reproductive limitations. In *S. typhle*, 1-year-old individuals are size monomorphic (Berglund et al. 1986a, b), but because males grow more slowly than females (Svensson 1988), 2-year-old males are smaller than females of the same age.

The aim of this study was to see: first, if the inclusion of older animals in the investigation could affect the earlier result that males limit female reproductive success (Berglund et al. 1989) and second, if the increase in sexual size dimorphism with age in this species is associa-

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ted with changes in the pattern of reproductive limitations. For this purpose, we performed an experiment to examine the capacity for different sized males and females to reproduce during a breeding season.

Relevant natural history

Males in all syngnathids (pipefishes and seahorses) provide their offspring with nutrients and oxygen while brooding them (Kronester-Frei 1975; Haresign and Schumway 1981; Berglund et al. 1986a). *Syngnathus typhle* inhabits meadows of *Zostera marina* (L.) along the coasts of Europe (Fiedler 1954; Kähnsbauer 1974; Wheeler 1978) where it is a visually guided predator, mainly on small crustaceans. It lives up to 3 to 4 years. The male possesses a brood pouch that closes within a couple of days during gestation; his body size correlates positively with number of offspring, size of offspring, and energy provided per offspring. In females, number of eggs and egg size are positively correlated with body size. Males receive eggs to fill their brood pouch from one or several females, and a female may divide one batch of eggs among several males (Berglund et al. 1988). Both sexes prefer to mate with large partners. This species is not sexually dichromatic, and behavior during the nuptial dance preceding copulation does not seem to differ between the sexes. In nature, the sex ratio is equal. (Unless ortherwise indicated, the information above is taken from Berglund et al. 1986a, b.)

Methods

Pipefishes were caught in shallow *Zostera* beds in the Gullmar Fjord on the Swedish west coast (58°15'N, 11°28'E). Females were caught before the onset of the breeding season to ensure measurement of their full reproductive potential. Two size classes of males and females were used, small and large. These size classes roughly correspond to 1- and 2-years-old animals, respectively, in natural populations (Table 1). The two size classes were chosen to represent the two size classes found in natural populations at the onset of the experiment, with the female size classes divided at 175 mm and the male size classes at 160 mm. Age classes were inferred from recruitment and size distributions throughout the year in natural populations: we know the size of newborns, and can precisely follow the growth of this cohort during autumn. During winter,

growth ceases, so 1-year-old individuals are readily discerned the next spring as the size distribution is extremely bimodal at this particular time. At the onset of the reproductive season, there were no significant length differences among the four small-body size groups (i.e., small males and females from the experiment and from simultaneously sampled field populations). The next longer homogeneous group was large males, where no difference between natural and experimental males was found. Large females make up the longest homogeneous group, where again natural and experimental females did not differ in length (Table 1).

The experiment lasted from 25 May to 24 August 1988 and was performed in 25 barrels (capacity 225 l) equipped with plastic *Zostera*, and with continuously renewed sea water (temperature 7°–21° C and salinity 25‰–33‰, following natural conditions). A natural light regime was followed. Fishes were fed in excess with live *Artemia*, small wild-caught crustaceans, and frozen mysids. Barrels were cleaned from leftover food and faeces every second day.

One female was placed in each barrel and throughout the experiment provided with three males carrying no offspring, but with well-developed brood pouches. Large females were provided with large males, and small females with small males. Thus, availability of mates did not limit female reproductive success in this experiment, allowing us to measure both the maximum number of males a female can fill to 100% of their capacity and the maximum egg production rate. This figure can then be compared to what she might achieve with naturally available mates, i.e., on average only one, as sex ratios are equal (Berglund et al. 1986a, 1989). Standard length was measured initially, and males were inspected every day for presence of embryos. If a male contained embryos, his standard length was measured, and the fullness of his brood pouch was estimated, after which embryos were carefully removed from the lightly anesthetized male (MS-222 solution). Fullness of the brood pouch is easily estimated because the pouch is filled from the rear forwards; thus part of the pouch remains empty until 100% fullness is reached. The pouch fullness was estimated to the nearest 10%. Embryos were counted and their dry weight (24 h; 60° C) measured. Emptied males were allowed to remate with their old females for practical reasons (we had a limited supply of males without embryos). The males freely remated soon after embryos were removed. Thus, females had continuous access to three receptive males.

Length of pregnancy (interval between last copulation and onset of hatching) was recorded in some of the males. These males were completely filled in the laboratory and subsequently kept under similar conditions as the experimental animals.

In females, standard length was measured at the beginning and at the end of the experiment. In addition, female body dry weight and number of mature eggs remaining in the overay were measured at the end of the experiment. A female's breeding period was considered to span the dates of the first and the last copulation.

The number of males one female could fill completely was estimated by summing the proportions of the brood pouches filled

Table 1. Body length in *Syngnathus typhle* from a natural population (May 1988), and from the reproductive-capacity experiment. A Scheffe range test was performed on a one-way ANOVA ($F = 522$, $P < 0.001$)

Size	Sex	Origin	Mean body length (mm)	n	95% Scheffe confidence interval	Homogeneous groups
Small	♂	nature	124.0	123	120.2–127.7	●
Small	♂	experiment	130.2	28	122.3–138.1	●
Small	♀	nature	124.9	113	121.0–128.9	●
Small	♀	experiment	130.5	11	118.0–143.1	●
Large	♂	nature	188.7	103	184.5–192.8	●
Large	♂	experiment	189.5	72	184.6–194.4	●
Large	♀	nature	211.9	150	208.5–215.3	●
Large	♀	experiment	220.4	11	207.8–232.9	●

with embryos of every male with which she had mated. This was done for two periods of time: the average duration of a pregnancy and for the entire reproductive season.

Egg surplus was also estimated as follows. The average number of embryos a full male (of the same body length as the female) contained was subtracted from the number of eggs spawned by the female within the average duration of one male pregnancy, the difference representing the egg surplus. The number of embryos in full males was calculated from a regression of embryo number on body length based on field samples from 1983 and 1984 (combined).

Test probabilities reported here are two-tailed.

Results

Pregnancy duration

The pregnancy in *S. typhle* males averaged 36 days (SD = 7, n = 11). Male size was not correlated with duration of pregnancy (r = 0.47, n = 11, P = 0.15), and hence there is no need to compensate for differences in pregnancy duration when comparing large and small males.

Numer of filled males and egg surplus within one male pregnancy

Within the time span of an average pregnancy (36 days), a *S. typhle* female (large and small averaged) copulated 3.4 times (SD = 2.0, n = 21), providing each mate with, on average, 53% (47 eggs) of a full pouch. Number of copulations was positively correlated with female body length (r = 0.62, n = 21, P < 0.003). Within this time span, an average female would fill 1.8 male brood pouches to 100% (SD = 1.3, n = 21), a number significantly greater than 1 (t = 3.07, P < 0.007). Number of completely filled males also correlated with female body length (r = 0.674, P < 0.001). If instead, we compare number of males filled by large and small females, respectively, within 36 days, large females would fill on average 2.7 large males to capacity (SD = 0.95, n = 10), whereas small females would fill 1.06 small males (SD = 0.99, n = 11), which constitutes a significant difference (t = 3.88, P = 0.001).

Another estimate of egg surplus in females is to compare the number of eggs spawned by one female within the time span of one pregnancy with the number of eggs needed to fill one similarly sized male (Berglund et al. 1989). This is a conservative estimate because in nature (and in the experiment) 2-year-old males were actually smaller than 2-year-old females. Hence, the egg surplus of 2-year-old females was underestimated. By this method, egg surplus (average of large and small females) was 65 eggs (SD = 112, n = 21), significantly more than no surplus (t = 2.67, P < 0.02). Furthermore, egg surplus was correlated with female body length (r = 0.70, P < 0.001). When large and small females are separated, we find that large females produced a surplus of 149 eggs (SD = 107, n = 10), significantly more than no surplus (t = 4.40, P < 0.002), whereas small females had no surplus (average −10 eggs, SD = 41, n = 11, t = 0.85, P =

0.41). Thus, larger females produced a greater egg surplus than small females (t = 4.59, P < 0.001).

Reproduction throughout the breeding season

During the course of the experiment, spanning the breeding season, females (large and small averaged) copulated with 4.4 males (SD = 3.1, n = 23), and deposited in them enough eggs to completely fill 2.4 males (SD = 2.0, n = 23). Large females filled more large males to capacity during the whole season compared to the number of small males filled by small females (large females: 3.5 males, SD = 1.9, n = 12; small females: 1.2 small males, SD = 1.2, n = 11; t = 3.54, P < 0.002), and also transferred more eggs per copulation (large females: 56 eggs, SD = 39, n = 72; small females: 23 eggs, SD = 15, n = 28; t = 4.37, P < 0.001). If we allow for differences in male length, however, female size did not influence the number of eggs transferred per copulation (multiple regression analysis, P = 0.93), whereas the reverse was true: larger males received more eggs per copulation, independent of female size (P < 0.001). The length of the reproductive season also differed between large and small females: large females reproduced during 55.7 days (SD = 21.5, n = 9) and small females during 16.5 days (SD = 16.6, n = 11; t = 4.59, P < 0.001).

Female body length, furthermore, was positively correlated with total number of eggs produced (spawned + remaining at the end of the experiment, r = 0.81, n = 23, P < 0.001).

Male body length was positively correlated with number of eggs received per copulation (r = 0.51, n = 93, P < 0.001), clutch dry weight (r = 0.36, n = 42, P < 0.02), and egg dry weight (r = 0.38, n = 42, P < 0.02). Egg dry weight also increased towards the end of a female's reproductive period (average increase 0.153 mg, SD = 0.20, n = 10; the increase differed significantly from zero: t = 2.41, P < 0.05). Male body length was not significantly correlated with degree of pouch fullness (r = 0.15, n = 93, P < 0.1).

Discussion

Our experiment demonstrates that small 1-year-old females on average needed one similarly sized male to accommodate all the eggs she could produce, whereas large, 2-year-old females needed 2.7 similarly sized males to harbor their eggs. Does this mean that in nature the reproductive success of a small female is not constrained by mate availability, whereas that of a larger female is?

In our experimental design, small females exclusively mated with small males, and large females exclusively with large males. We know, however, that males prefer to mate with larger females because they produce larger eggs (Berglund et al. 1986b) and transfer more eggs per copulation; we also know that natural sex ratios are equal (Berglund et al. 1986a, b, 1988). Because we demonstrated here that an egg surplus in the population

as a whole exists, the following conclusions seem inevitable: females will mate with any available male, whereas male mate preferentially with larger females. If large females make up a substantial part of the population, small females can expect to engage in few matings, and will hence be limited by male availability. Even if large females can monopolize all males, they too will probably still be limited by male availability: they need 2.7 similarly sized males, but can on average expect to mate with only one similarly aged, and thus slightly smaller, male, and of some fraction of the small 1-year-old males. Most likely this will not meet their demands.

A mating pattern where large females monopolize all males is not possible in this species, however: that requires exclusive territories or leks, which are not found here (personal observation), and monogamous pairs are not formed, in contrast to the case in an Australian pipefish (Gronell 1984). At any rate, large females probably engage in a disproportionately large amount of matings, and hence the value of producing eggs will decrease for small females. In a mating experiment, females first mated with the largest available male, but did not differentiate between empty and half-filled males (Ahnesjö, in preparation).

A mating hierarchy may exist among males, but even if so, that is unlikely to change the limitation pattern outlined above: once a dominant, and presumably large, male has received a full pouch, he will gain nothing by refraining other males from mating; however, he still has to face the costs associated with being dominant.

Large females begin reproducing earlier in the season than small ones; therefore, they initially may not be limited by mate availability. The same temporal pattern occurs in males, however: large males begin reproducing earlier than small ones, with the result that only small males are available at the onset of the small females' reproductive period. In addition, large females filled on average 3.5 males per summer, whereas small females filled only 1.2. Furthermore, large females are more fecund, have a longer reproductive season, and fill more males throughout the reproductive season than small females. Hence, the male-imposed constraints on female reproduction probably affects small females more, forcing these to terminate reproduction relatively early (this has also been independently confirmed; Berglund, in preparation).

If the effects of body size are ignored, our results agree with those obtained previously (Berglund et al. 1989). In the earlier study, S. typhle females filled on average 1.9 males, and here 1.8 males. Similarly, egg surplus in the previous experiment was 55 eggs, here 65 eggs. Duration of pregnancy was shorter in the present experiment (probably due to higher water temperatures), and average body lengths were larger because 2-year-old individuals now were included. The 1-year-old cohort was shorter here, however, probably due to slow juvenile growth in the unusually cold 1987 summer.

To conclude, we found that small females need on average one male to accommodate their eggs, whereas large females need 2.7 males. The reproductive success of small females is nevertheless severely constrained by male availability because males prefer to mate with large females, which thus gain access to a disproportionately large share of the male pool. Hence, small females should be expected to show a reduced reproductive effort and instead allocate part of the available resources to somatic growth, thus becoming more attractive in their second year of life. This assumes a trade-off between reproduction and growth and predicts an adjustment of the reproductive expenditure in small females as a direct response to the presence of large females. Both the assumption and the prediction have been investigated and verified (Berglund, in preparation).

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