Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback

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An increasing number of studies show that animals adjust their reproductive effort to the risk of predation. However, to maximize lifetime reproductive success this adjustment should depend on the animals' current and future reproductive potential. Here I tested this hypothesis by allowing threespine stickleback males (*Gasterosteus aculeatus*), differing in current and future mating probabilities, to reproduce in pools in both the presence and absence of predators. As expected, males adjusted their reproductive effort to the risk of predation. Fewer males bred, and all males developed less nuptial coloration in the presence of predators. However, males with a low current mating probability took less risk than males with a higher mating probability, whereas all males increased risk taking when future reproductive opportunities decreased. The results thus support the hypothesis that males are able to assess both the risk of predation and their current versus future mating probability, and adjust their reproductive decisions accordingly. The study further suggests that predation risk may have less effect on sexual selection than previously assumed, as the males which refrained from reproducing in the presence of predators were mainly males with a low mating probability.

Keywords: reproduction; predation risk; mating probability; nuptial coloration; threespine stickleback

1. INTRODUCTION

Investment into current reproduction may incur a cost in the form of reduced future fecundity, due to the exhaustion of energy and nutrients, or endangered survival and future reproduction (Williams 1966; Bell & Koufopanou 1986). Recently, predation risk has received increased attention as a cost of reproduction. Reproducing animals are often exposed to increased predation risk owing to an increased conspicuousness to predators, a decreased possibility of escape, or more frequent predator encounters (Magnhagen 1991). Consequently, animals should balance their reproductive decisions, i.e. when, where and how to reproduce, against predation risk so that their lifetime reproductive success is maximized (Lima & Dill 1990; Magnhagen 1991). In support of this, predation risk has repeatedly been found to be a strong selective force in the evolution of reproductive adaptations (Sih 1987; Magnhagen 1991).

The risk of predation is, however, seldom constant but varies in time and space during an individual's lifetime. Animals should therefore also evolve an ability to assess short-term changes in predation risk and adjust their reproductive decisions accordingly (Lima & Dill 1990). Evidence that animals are able to adjust their reproductive decisions to the prevailing predation risk is currently growing. Animals have been found to suppress reproduction under increased predation risk by refraining from reproducing (Magnhagen 1990; Ylönen 1994) or by decreasing investment into reproduction, e.g. the amount of eggs laid (Fraser & Gilliam 1992; Wisenden 1993) or

the intensity of courtship (Berglund 1993; Forsgren & Magnhagen 1993; Godin 1995, and references therein).

Few studies have, however, investigated whether animals try to maximize their lifetime reproductive success by adjusting their reproductive decisions and risk taking to their expectations of current and future mating. According to a model by Kâlâs *et al.* (1995), present mating probability should affect risk taking. Decreasing residual reproductive value (RRV) should again increase investment in current reproduction (e.g. Williams 1966; Pianka & Parker 1975; Sargent & Gross 1986).

Here I investigate whether reproductive effort under predation risk depends on prospects of current and future mating in the threespine stickleback (*Gasterosteus aculeatus*). Reproducing stickleback males are more vulnerable to predators than non-reproducing ones, owing to their bright, red, breeding coloration and their conspicuous reproductive activities (Moodie 1972; Whoriskey & FitzGerald 1985), and are therefore expected to balance their investment in current reproduction against predation risk. I allowed males, differing in current and future mating probabilities, to reproduce in both the presence and the absence of predators. Current mating probabilities were defined by body size. Large males were expected to have a larger mating probability than smaller males as females are known to prefer larger males (Moodie 1982; Rowland 1989). Future reproductive opportunities were defined by the time of the breeding season. In the present study population the males' expected future reproductive opportunities decrease as the season progresses, as the males have a single breeding

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season during which they reproduce repeatedly (Virtanen 1988).

2. METHODS

I collected threespine stickleback males twice a week with fry traps from the littoral of the Baltic Sea near Tvärminne Zoological Station in south-western Finland. Males were held in the laboratory in brackish water storage tanks (salinity 5.5 p.p.t.) on an 18:6 h light–dark cycle at 15 °C. They were fed twice daily in excess with commercial flake food and chironomids. Breeding behaviour was discouraged by the lack of suitable nesting materials and the high density of fish.

After 3–5 days of storage, six sexually mature males with blue eyes and a hint of red around their mouths were placed into each of four outdoor wading pools (1.4 m in diameter, water depth 30 cm). Each pool had a glass aquarium (50 cm × 30 cm × 30 cm) in the middle. Two of the pools served as predator treatments with two natural predators, perch (*Perca fluviatilis*, 21–25 cm standard length (SL)), in the central aquaria. The two other pools served as controls, with the central aquaria left empty. Fresh seawater was continually flowing through the glass aquaria into the pools. Thus, sticklebacks could both see and smell the predators. Each pool had ten bunches of artificial vegetation, and the bottom was covered by sand and tufts of *Cladophora*, which served as nesting material. The six males in each pool ranged in size from 44 to 60 mm SL (mean size±s.d.=52.6±2.2 mm). The size distribution was about the same in each pool, as each pool contained three small males, 44–50 mm, and three large males, 55–60 mm.

The males were left for 2 d to establish territories and build nests. On the third day, six females which, judging from the extension of their bellies and the opening of their cloacae were ready to spawn, were added to each pool. Stickleback males may mate with several females and only the male cares for the eggs. On the following day, at midday, the effect of predation risk on the males' reproductive decisions was determined. First, the intensity of the red breeding coloration and the possession of a territory and a nest were determined for each male by observing them while they were undisturbed in the pools. The red breeding coloration intensifies during breeding activities and affects female mate choice (reviewed by Rowland (1994)). A scale from 1 to 5 was used for colour scoring, class 5 showing the brightest coloration (corresponding to the scale described by Rowland (1984)). Territory diameters were estimated by observing the males while they were defending their territories, i.e. by determining the boundaries of their territories. To test the repeatability of the colour scoring method, one randomly chosen male was hand-netted from each undisturbed pool and photographed under standardized conditions, i.e. the male was placed in a small glass box (6 cm × 3 cm × 6 cm) containing water and a black sponge as a background and photographed in a dark box with a flash as the only light source. The males did not fade as the procedure took less than 1 min. Later, when the experiment was finished, the males were assigned a new colour score on the basis of the photographs, and the score was compared with the one assigned in the pools. Finally, the rest of the males were caught and measured for body length and the volumes of eggs in the nests were recorded.

To investigate the effect of decreasing future reproductive opportunities on reproductive effort the experiment was replicated at seven different times of the season. New, recently caught fish were used each time, and a complete water exchange
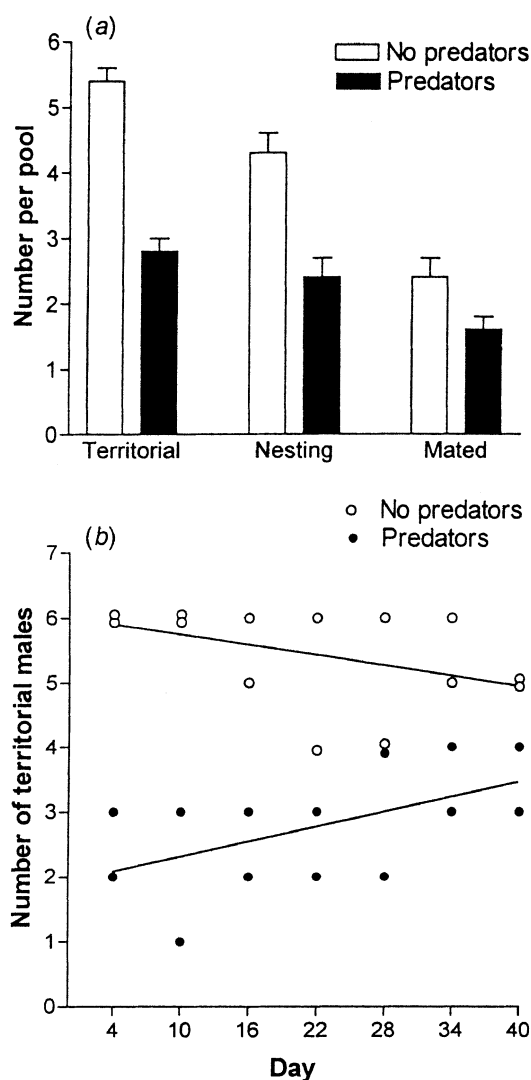
Figure 1. (*a*) Mean number (+ s.e.) of territorial males, nesting males (i.e. territorial males with a nest) and mated males (i.e. nesting males that have mated) in the absence and presence of predators. (*b*) Number of territorial males as a function of time of the breeding season in the absence ($y=6.07 - 0.16x$) and presence ($y=1.86 + 0.23x$) of predators. Each record represents one replicate. Day 1=1 June.

was done between replicates. The mean length of all males in each pool did not differ between treatments or in time (predators, $F_{1,14}=0.62$, n.s.; time, $F_{1,14}=1.18$, n.s.; predators × time, $F_{1,14}=0.39$, n.s.). The experiment ran from 1 June to 10 July 1996. In natural conditions, breeding males were found from the end of May to the end of July.

All analyses are based on pool means to eliminate the possibility of pseudoreplication. Territory size and egg volume were log-transformed to equalize variances.

3. RESULTS

(a) *Number and size of males attempting to reproduce*

Predator presence decreased the number of males attempting to reproduce (figure 1*a*). However, there was a significant interaction between the effect of predators and time of the season (two-way MANOVA: predator
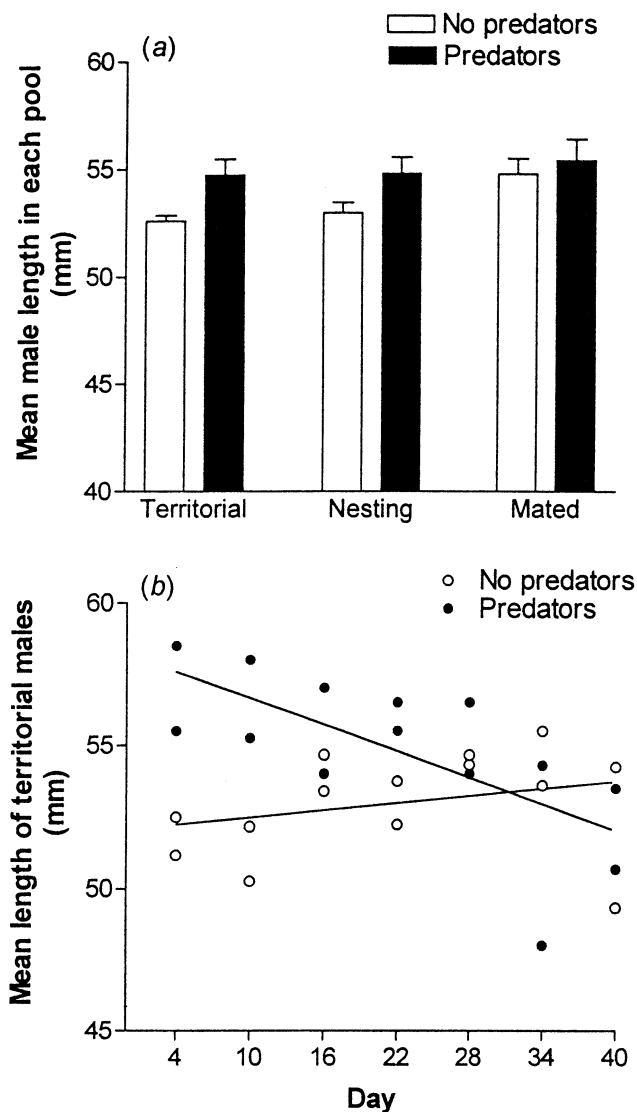
Figure 2. (*a*) Mean body length (+ s.e.) of territorial, nesting and mated males in the absence and presence of predators. (*b*) Mean length of territorial males as a function of time of the breeding season in the absence ($y = 51.98 + 0.04x$) and presence ($y = 58.5 - 0.15x$) of predators. Each length record is the mean value of each replicate. Day $1 = 1$ June.

treatment, Wilks's lambda $= 0.28$, $F_{3,22} = 18.51$, $p < 0.001$; time, Wilks's lambda $= 0.74$, $F_{3,22} = 2.64$, $p = 0.075$; predator $\times$ time, Wilks's lambda $= 0.55$, $F_{3,22} = 5.98$, $p < 0.01$), i.e. the suppressive effect of predators decreased towards the end of the season (figure 1*b*).

Males attempting to reproduce were, on average, longer in the presence of predators (*t*-test for unequal variances: territorial, $t_{16.3} = 2.70$, $p < 0.05$; nesting, $t_{22.2} = 2.04$, $p = 0.05$; figure 2*a*). Thus, mainly small males refrained from reproducing when predators were present. As expected, small males had a lower mating probability than large males in the absence of predators, as mated males were on average longer than unmated nesting males (mean SL $\pm$ s.e. $= 54.8 \pm 0.71$ mm and 51.8 ± 0.71 mm, respectively; paired $t_{12} = 3.01$, $p < 0.05$). In the presence of predators there was no difference in size between mated and unmated nesting males (paired $t_6 = 0.17$, n.s.). This is due to the decreased possibility for female mate choice for size in the presence of predators when mainly large males nest

and the size distribution of nesting males is more even (coefficient of variance (CV) in body length: no predators, mean $\pm$ s.e. $= 10.6 \pm 0.66$; predators, 7.4 ± 1.45; $F_{1,24} = 4.54$, $p < 0.05$).

The mean length of territorial males decreased over the season in the presence of predators, but was unchanged in the absence of predators ($r^2 = 0.47$, $F_{1,12} = 10.65$, $p < 0.01$ and $r^2 = 0.08$, $F_{1,12} = 1.10$, n.s., respectively; figure 2*b*). Thus, the suppressive effect of predators on current reproductive effort of small males decreased towards the end of the breeding season.

(b) *Nuptial coloration and territory size*

The colour scores assigned to photographed and free-swimming males were significantly correlated ($r_s = 0.81$, $n = 28$, $p < 0.001$). As all photographs were evaluated at the same time, the scoring method used seems reliable and repeatable.

Males developed less red breeding coloration in the presence of predators (Mann–Whitney *U*-test, $n = 28$: non-territorial, $z = 3.34$, $p < 0.001$; territorial, $z = 2.94$, $p < 0.01$; nesting, $z = 3.24$, $p < 0.01$; mated, $z = 3.06$, $p < 0.01$; figure 3*a*). However, the suppressive effect of predators on nuptial coloration decreased over the season as male coloration increased over time in the presence of predators (non-territorial, $r_s = 0.52$, $p < 0.1$; territorial, $r_s = 0.83$, $p < 0.001$; nesting, $r_s = 0.83$, $p < 0.001$; mated, $r_s = 0.80$, $p < 0.001$) but not in the absence of predators (non-territorial, $r_s = -0.14$, n.s.; territorial, $r_s = 0.44$, n.s.; nesting, $r_s = 0.40$, n.s.; mated, $r_s = 0.34$, n.s.; figure 3*b*).

Territory size was not affected by predator presence or time when adjusting for the number of territories (two-way ANCOVA: predators, $F_{1,13} = 0.17$, n.s.; time, $F_{1,13} = 1.36$, n.s.; predators $\times$ time, $F_{1,13} = 1.03$, n.s.; number of territories, $F_{1,13} = 8.76$, $p < 0.05$). However, the variation among nesting males in territory size (CV) depended on time (predators, $F_{1,13} = 2.17$, n.s.; time, $F_{1,13} = 2.79$, $p < 0.05$; predators $\times$ time, $F_{1,13} = 0.35$, n.s.), i.e. it decreased over the season ($r^2 = 0.33$, $b = -5.28$, $F_{1,26} = 12.50$, $p < 0.01$).

(c) *Male mating success*

Predator presence or time did not affect the males' mating success, i.e. the amount of eggs received, when adjusting for the number of nests (two-way ANCOVA: predators, $F_{1,13} = 0.04$, n.s.; time, $F_{1,13} = 1.59$, n.s.; predators $\times$ time, $F_{1,13} = 0.57$, n.s.; number of nests, $F_{1,13} = 10.55$, $p < 0.001$). Females always shed all of their eggs and the total amount of eggs spawned was unaffected by predator presence and time (predators, $F_{1,14} = 1.98$, n.s.; time, $F_{1,14} = 0.60$, n.s.; predators $\times$ time, $F_{1,14} = 0.28$, n.s.). However, the variation among nesting males in the amount of eggs received decreased over the season ($r^2 = 0.36$, $F_{1,24} = 13.6$, $p < 0.01$; figure 4). This could be due either to reduced female choosiness over the season or to a reduced difference among males in traits that affect female mate choice, decreasing the opportunity for female mate choice.

4. DISCUSSION

The presence of predators affected reproductive effort of threespine stickleback males. Fewer males established territories and constructed nests in the presence of
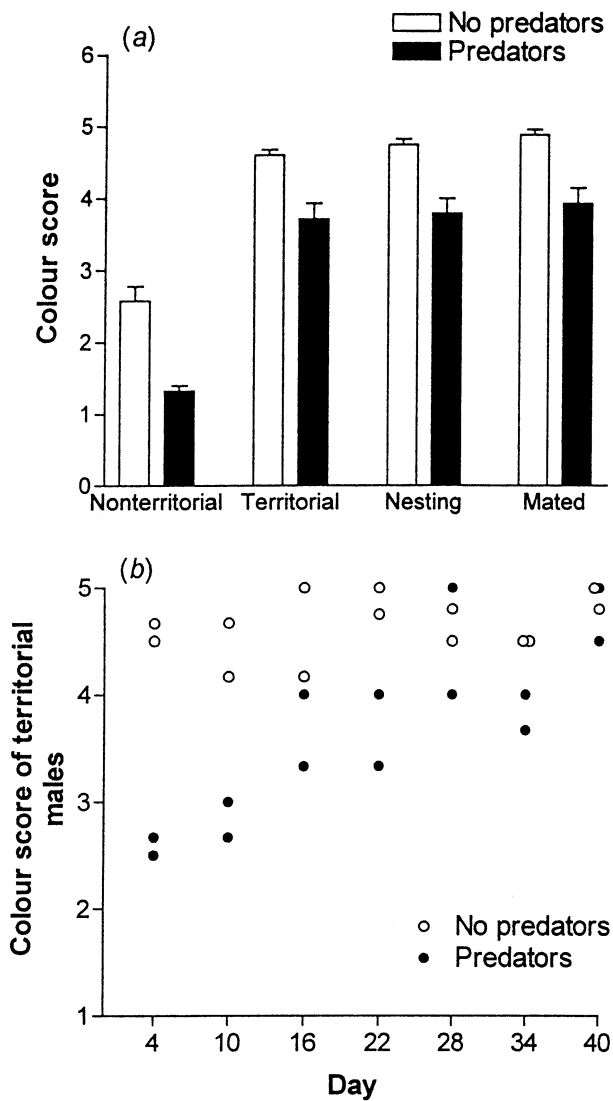
Figure 3. (*a*) Mean colour scores (+ s.e.) of territorial, nesting, and mated males in the absence and presence of predators. (*b*) A scatter plot of nuptial coloration of territorial males against the time of the breeding season in the absence and presence of predators. Each colour score is the mean value of each replicate. Day 1 = 1 June.
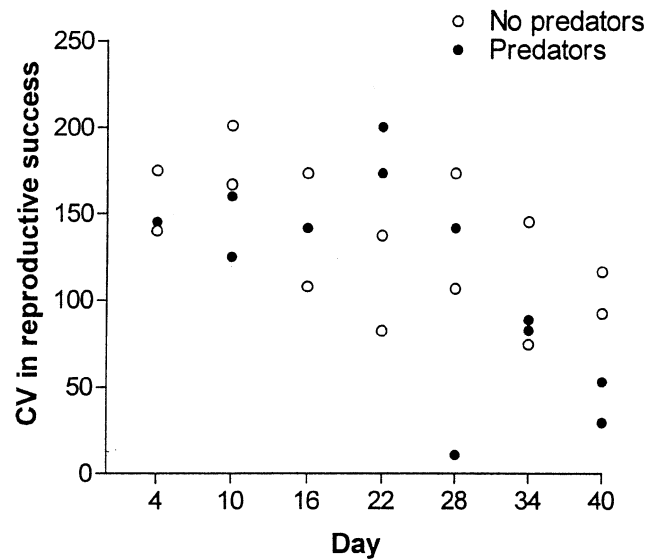

Figure 4. Variation in reproductive success (CV of egg numbers) among nesting males in each pool as a function of time of the breeding season ($y = 186.4 - 14.9x$). Day 1 = 1 June.

predators, and both territorial and non-territorial males invested less in a conditional secondary sexual character, nuptial coloration, which is known to play a crucial part in both male–male competition and female mate choice (reviewed in Rowland (1994)). The decreased reproductive effort in the presence of predators could be either a direct response to predator presence, or a consequence of an increased investment in anti-predator behaviour, i.e. less time and energy being available for reproduction. Alternatively, reduced current reproductive effort could be indirectly induced by predator-mediated changes in female behaviour. This explanation seems, however, less likely as predators did not alter the decision of females whether to spawn or not, and the females were only present during the last day when the males had already established territories, built nests and developed nuptial coloration.

By reducing current reproductive effort when predation risk is high, individuals may decrease their current reproductive success but increase their probability of survival and future reproduction. However, to maximize

lifetime reproductive success, this adjustment should depend on prospects of current and future mating. This study strongly indicated that this is the case. Small males with a low current mating probability refrained from attempting to reproduce more often than larger males with a higher mating probability when predators were present, but increased risk taking when future reproductive opportunities decreased. The lower reproductive effort of small males could be argued to be due to small males experiencing higher risks, owing to their smaller body size. However, this seems highly unlikely in this study as larger threespine stickleback males have been found to be preferred by fish predators (Külling & Milinski 1992), and perch of the size used in this experiment have been found to catch preferentially large males in the present area (U. Candolin, unpublished data). However, the current mating probability of small males could increase towards the end of the season and contribute to their increased risk taking. Large males could be in a poorer condition towards the end of the season, owing to their larger investment in reproduction early in the season. This could increase the competitive advantage and mating probability of small males. In support of this, the variation among nesting males in mating success decreased over time in both the presence and absence of predators. This was probably due to a decreased difference among males in traits that affect mating success, as female choosiness is expected to increase late in the breeding season in species that reproduce repeatedly during a single breeding season (Crowley *et al.* 1991), and the variation among males in territory size, which is positively correlated with mating success (Goldschmidt & Bakker 1990), decreased over the season.

However, the effect of future reproductive opportunities on risk taking is further supported by the decreased effect of predators on nuptial coloration towards the end of the season. Indications of age-dependent risk taking during reproduction have been found in the black goby, *Gobius*

niger (Magnhagen 1990), and parental investment has been shown to depend on expectations of future mating in the common goby, *Pomatoschistus microps* (Magnhagen & Vestergaard 1991), and in some stickleback populations (reviewed in Whoriskey & FitzGerald (1994)). However, the results in this study are especially intriguing as males should be expected to be in a poorer condition towards the end of the season and thus have less resources to invest in reproduction.

The effect of predators on reproductive effort has been assumed to have significant effects on sexual selection by affecting female mate choice (reviewed by Andersson (1994) and Godin & Briggs (1996)). In the case of the stickleback, the lower investment into nuptial coloration in the presence of predators could affect the males' relative attractiveness to females and thus decrease the ability of females to discriminate among males. As nuptial coloration is known to reflect male condition (Milinski & Bakker 1990), this could equalize the mating success among males of different quality. In addition, the decrease in the red coloration could decrease the distance at which females can detect males, and thus reduce the number of detected males and the opportunity for female mate choice. However, as males with a low mating probability, which most likely are low-quality males, did not attempt to reproduce in the presence of predators, the need for careful female mate choice should be reduced. Thus, presuming that high-quality males do not suffer from a markedly increased mortality, sexual selection could be less affected by predator presence than previously assumed.

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