

# Sexual selection in an anadromous population of threespine sticklebacks – no role for parasites

GERARD J. FITZGERALD\*, MARC FOURNIER and JULIE MORRISSETTE

*Département de Biologie, Université Laval, Ste-Foy, Québec, Canada, G1K 7P4*

## Summary

We examined the hypothesis that trematode parasites played a role in the evolution of the red colour of male threespine sticklebacks (*Gasterosteus aculeatus* L.) and whether the parasites affected female fitness. Parasites ('blackspot' disease) played no role either in determining the outcome of male–male competition for breeding territories or in female mate choice. Among males with territories, mating success was highly variable. Some males obtained over 3000 eggs (approximately 10 matings) whereas others received none. In 1 year of the 2 year study, males with the greatest amount of red nuptial coloration had the greatest mating success. Although male colour may sometimes affect female choice in this system, this preference has probably not evolved because of the Hamilton–Zuk mechanism of sexual selection. The parasites had small, but statistically significant effects on female fitness. Females with high parasite loads were in poorer condition and produced fewer eggs than less parasitized fish.

**Keywords:** mating success; nuptial coloration; parasites; sticklebacks

## Introduction

Selection for traits which are solely concerned with increasing mating success is usually referred to as sexual selection. Sexual selection can work either by favouring the ability of one sex, usually males, to compete directly with another for fertilizations (e.g. by fighting) or by favouring traits in one sex which attract the other. The two mechanisms are not mutually exclusive. Perhaps the most controversial aspect of sexual selection theory is whether a female's choice of traits is arbitrary or adaptive. For example, if the choice is arbitrary, females who choose males with brightest colours or biggest tails get nothing from the interaction other than the production of male offspring with the preferred trait (Fisher, 1930). The choice of an epigametic trait is adaptive if there is an association between the expression of the trait and male viability. For example, females who choose males with bigger tails or brighter colours may get 'good genes' and, thus, produce offspring who possess the preferred trait and are more vigorous than offspring of non-preferred males. Unlike the non-adaptive Fisherian hypothesis, the 'good genes' hypothesis unites superiority in attracting mates and superiority in survival ability into a mutually reinforcing force of intersexual selection (McLennan and Brooks, 1991).

However, a potential problem with the 'good genes' hypothesis is how genetic variability for the preferred trait is maintained in the population if female choice of the male with the best genes is a strong enough directional force to influence evolutionary elaboration of the character (Maynard Smith, 1985). Hamilton and Zuk (1982) suggested that parasites may be a mechanism for maintaining variation for good genes. If parasites and their hosts are involved in a co-evolutionary arms race, the time lag between changes in characters involved in parasite offence

\*To whom correspondence should be addressed.

and host defence produces cycles of co-adaptation. This temporally varying selection pressure maintains a significant level of additive genetic variation for viability in the host population. For the proposed mechanism to work, the parasites must affect the health (viability) of their hosts, the condition of the male's epigamic characters must be an accurate reflection of the bearer's health – a revealing handicap – and the parasite resistance has to be genetically determined.

Evidence for and against the Hamilton–Zuk hypothesis is slowly mounting. However, there are relatively few field studies supporting the hypothesis, but even fewer that do not support it (see Read, 1988; McLennan and Brooks, 1991). Recently, Milinski and Bakker (1990) showed that when female threespine sticklebacks, *Gasterosteus aculeatus*, were presented in the laboratory with a choice between two nuptially-coloured males, they were more attracted to the brighter (redder) one. When these bright males were experimentally infected with a cilian parasite, *Ichthyophthirius multifiliis*, that causes 'whitespot' disease, the infected males lost most of their red coloration, and those that had been previously accepted by the females were now spurned. Milinski and Bakker's (1990) results argued that a male's coloration might be a revealing handicap for his condition because a significant positive correlation was found between the body condition and male coloration and the correlation decreased when males were experimentally infected by parasites. They speculated that females should prefer the strongest males because these males will be best equipped to survive the rigours of parental care. Females could also simultaneously be selecting genes favourable for parasite resistance if there is additive genetic variation for parasite resistance in the population. This scenario, while reasonable, needs to be verified for females choosing males under field conditions. They gave no evidence of natural levels of infestations of 'whitespot'. In nature, other factors may be more important than nuptial coloration or parasites in female mate choice.

There have been relatively few studies of factors determining the mating success in sticklebacks in the field (see Whoriskey and FitzGerald (1993) for a review) and no field studies to evaluate the possible role of parasites in sexual selection processes. Our first objective was to determine if there is any association between the degree of nuptial coloration, parasite load, physical condition and male mating success in a population of anadromous sticklebacks. Our second objective was to ascertain whether parasites affected male parental care, because in this species males are the exclusive care-givers.

Parasites could affect a male's mating success if heavily parasitized males are unable to obtain a breeding site or if females could avoid heavily parasitized males. Male parental care could be affected if heavily infected males were unable to meet the rigours associated with defending the young against predators. Parasites could affect female fitness by reducing their egg production or their post-breeding season survival relative to uninfected fish.

We addressed the following questions.

(1) Is a male's parasite load related to its chances of obtaining a territory? Except for a minority of males that sneak fertilizations (Rico *et al.*, 1992), territories are essential for male reproductive success. If parasites affect the outcome of male–male competition for territories, losers of fights for territories should harbour more parasites than winners.

(2) Do parasites affect a territorial male's mating success (number of eggs he receives)? If females avoid heavily infected males, then there should be a negative correlation between parasite load and mating success.

(3) Is there any association between nuptial coloration, parasite load and physical condition in territorial males? If parasites had a debilitating effect, heavily infested males should be duller and in poorer physical condition (Milinski and Bakker, 1990).

(4) Does parasite load affect parental care? Heavily parasitized fish might show low levels of care if the parasite is debilitating.

(5) Does parasite load affect female fecundity and physical condition?

## Methods

### *Study site*

Our study site was a 400 m<sup>2</sup> tide pool located in a saltmarsh situated near Isle-Verte approximately 200 km northeast of Québec City, Canada along the southern shore of the St Lawrence estuary (48° 01' N, 69° 21' W).

The pool was arbitrarily chosen among the several hundred available. In early May, threespine sticklebacks migrate from their overwintering grounds in the middle St Lawrence estuary into the marsh. All breeding takes place in May and June and the period of peak reproduction is between mid-May and mid-June.

Approximately 500 adult threespine sticklebacks of both sexes entered the pool on the spring tides in late May. Within 24–48 h, the males had established territories, built nests and courted females. The successful ones began caring for eggs within 48 h. All sampling of males and their nest contents took place from 27 May to 18 June 1991 and from 4 May to 18 June 1992.

### *Threespine sticklebacks*

Threespine sticklebacks are characterized by lone male care that consists mainly of aggressive defence of eggs and fry and aeration of the eggs. Females spend most of their time foraging for food either alone or in shoals usually in the offshore areas of the pools. Individuals of both sexes of our population can live 2 years. During the breeding season most populations are sexually dimorphic (Wootton, 1976). The males display a bright red nuptial coloration and blue eyes. However, there is considerable variation in the amount of red among males within a population. Some males have red extending from the tip of their snouts to their anal pores, whereas others display only a little red around the throat region (see below). Previous laboratory work on freshwater populations of this species indicated females usually prefer to mate with the reddest males (e.g. Semler, 1971; McLennan and McPhail, 1989; Milinski and Bakker, 1990). The male's degree of coloration may also play a role in male–male interactions. Bright males of a freshwater population were more likely to defeat dull males in dyadic fights (Bakker and Sevenster, 1983). However, this did not occur in an anadromous Dutch population (T. C. M. Bakker, personal communication) or for our anadromous males (FitzGerald and Kedney, 1987).

### *Blackspot disease*

'Blackspot' is a general term for a disease caused by infestation with trematode larvae of a variety of species, *Uvulifer*, *Crassiphilia*, *Ornithodiplostomum* and *Postodiplostomum* sp. The life cycle of these trematodes is complex and involves a series of hosts with fish serving as an intermediate step in the development of adult parasites. Infection occurs when a fish encounters a free-swimming cercaria that penetrates its skin, forms an encysted metacercaria and awaits the opportunity to pass to the final host when the fish is eaten. In response to the metacercarial cyst, the fish forms a capsule of connective tissue that contains melanophores. 'Blackspot' refers to the peppered appearance given to the fish by these capsules. Unfortunately, we were unable to identify the causative species of digenic trematode, however, for the purposes of this study, we assume that each black spot represented penetration by one cercaria (Lemly and Esch, 1984).

Although the impact of blackspot disease upon male threespine sticklebacks is unknown, this

disease has been reported to negatively affect growth and survival in several other fish species. For example, severe infections caused the death of killifish, *Fundulus diaphanus* and minnow, *Pimephales promelas*, in only several days (Hoffman, 1956). Less severe infections have caused important weight losses in the pumpkinseed sunfish, *Lepomis gibbosus* (McCoy, 1928), bluegill sunfish (Lemly and Esch, 1984) and in the smallmouth bass, *Micropterus dolomieu* (Hunter and Hunter, 1938).

#### Field protocol

To answer our study questions, we captured territorial males (1991 and 1992) and non-territorial males (1991 only) from within the same single tide pool during the breeding season. Territorial males were captured with a dip net while they were fanning (aerating) their nests. They are easily lured into the dip net by placing an intruder male inside a glass jar inside the dip net.

Non-territorial males were caught with minnow traps located in the centre of the pool. Non-territorial males usually associate with females in shoals in the centre of the pools. We collected approximately 50% of all the males that entered the pool in the 2 years.

Because coloration of preserved specimens fades rapidly, we immediately evaluated nuptial coloration upon capture. Males were assigned a score on a scale of 0–3 (0 = no colour, 1 = red only on the lower jaw, 2 = red on head to opercule and 3 = red to anal pore). Our method is similar to that of Rowland (1984), except he judged colour on a scale of 1–5. Colour evaluation was done by one observer (M. Fournier). Although some of our scores were for parental males, rather than courting ones, there is a strong positive correlation between the amount of coloration shown by an individual during its courtship and its parental phases (McLennan and McPhail, 1989). Therefore, we assume that when we collected a bright male it had also been bright during its courtship phase, relative to other males. Some authors (e.g. van Iersel, 1953), have reported that males are reddest when courting females. We have not found this to be the case with our fish, although males can rapidly lose colour if frightened and gain colour if they win a fight. Next the male and its nest contents, if any, were preserved together in 4% formaldehyde.

In 1992 only, prior to the collection of males and their nests, we evaluated the quality of parental care by recording the risk that males took when defending their nests and their aggressiveness to a conspecific intruder. For each male, we recorded the time (s) before a male returned to within 5 cm of its nest after being disturbed by the observer. We assume that a male that returns sooner took more risks than a male that was slower to return (Lachance and FitzGerald, 1992). To measure aggression, we scored the number of bites that males made on a nuptially coloured male conspecific intruder placed 10 cm from its nest during a 10 min test. The two tests were separated in time by at least 1 h. For additional details of the experimental protocol, see Lachance and FitzGerald (1992).

To examine for correlations between parasite load and female fecundity (clutch size) and physical condition, we collected 270 females at various times over the breeding season. All fish were preserved as for the males and later in the laboratory, fish were weighed and measured (total length, mm) in order to calculate Fulton's index ( $K = 100 W/L^{2.73}$ ). Parasite load was evaluated as described for the males.

#### Laboratory protocol

The level of parasite infestation was quantified by counting all of the black spots on each preserved male approximately 2 months after the specimens were collected. Care was taken not to have colour scores in sight when the parasites were counted. To determine if blackspot disease affected males' chances of obtaining a territory, we compared the number of parasites on our samples of territorial and non-territorial males. Mating success of the territorial males was scored

by counting all eggs in the 194 nests. For each territorial male, we measured the total length (mm) and the wet weight (g). These data were then used to calculate Fulton's physical condition index ( $K$ ) where  $K = 100 W/L^3$ . This index is routinely used by fish biologists as a crude measure of fish health. We validated the use of the method by verifying that the slope of the logarithmically transformed values of the wet weight and length measures was approximately 3, i.e.  $\log W = 3 \log L + a$ . The slopes were 2.89 and 3.19 in 1991 and 1992, respectively.

### *Dyadic fights for territories*

Several hundred males were collected from the Isle-Verte saltmarsh prior to the onset of the breeding season and transported to Laval University where they were housed in a 500 l tank. After a minimum of 1 week acclimatization, males were selected randomly and divided into two groups – 'heavily parasitized' and uninfected or 'lightly parasitized'. The main distinction between the two males was their number of parasites. We did not count the number of parasites on the fish before a fight to minimize stress, but we deliberately matched fish that appeared to be significantly different in parasite load. We staged dyadic fights by introducing two males into a neutral 4 l tank. Under these conditions males will fight and usually a winner is revealed within 30 min (FitzGerald and Kedney, 1987). Winners were defined as those who repeatedly attacked the other fish without receiving attacks; losers were confined to the upper layer of the tank. Males fought only once and we only used data on fights where there was a clear-cut winner within 30 min. The parasites were counted only when a clear winner was determined.

We also recorded the weight (g), total length (mm) and colour (described above) before the fight and calculated Fulton's index to confirm that the opponents were comparable, except for parasite loads.

### *Statistical analyses*

Because our data were not normally distributed and often variables could not be transformed, we used non-parametric tests. All statistical calculations were done with the statistical package Statview 512+ (Feldman and Gagnon, 1986).

## **Results**

### *Male-male competition and parasites*

In 1991, 95% of the 94 territorial and 94% of the 50 non-territorial males collected had at least one parasite. There were no significant differences between the number of parasites of the territorial and non-territorial males (median for territorial males = 5, range = 0–24; median for non-territorial males = 4.5, range = 0–101; Mann-Whitney  $U$  test,  $z = -0.27$ ,  $p = 0.78$ ). In 1992, 83 of the 100 territorial males had at least one parasite (median = 5, range = 0 – 28).

Of 47 fights staged, 31 resulted in a clear-cut winner and only these fights were analysed. None of the measured variables – parasite number, body size, physical condition, colour score – prior to the fight predicted the winner (Table 1).

### *Parasites, mating success, health and colour*

Among males with eggs in their nests, there was no association between the numbers of parasites a male harboured and the number of eggs in his nest (1991: Spearman rank-order correlation coefficient  $r_s = 0.15$ ,  $p = 0.25$ ,  $n = 52$ ; 1992:  $r_s = 0.29$ ,  $p = 0.048$ ,  $n = 47$ ). We also compared the numbers of 'blackspot' on males who had some eggs in their nests with those that had no eggs. There was no difference between the two groups (1991: males without eggs, median (range)

Table 1. Characteristics of winners and losers of 31 dyadic fights between male threespine sticklebacks

Characteristic	Winners	Losers	<i>p</i>
Number of parasites	7 (0–140)	2 (0–28)	0.50
Wet weight (g)	3.4 (1.7–3.9)	3.2 (1.8–4.0)	0.40
Fulton's index	0.88 (0.71–0.89)	0.89 (0.76–1.04)	0.37
Colour score	0 (0–3)	0 (0–3)	0.63

Data are medians (ranges). *p* = probability value (Wilcoxon signed ranks test).

Table 2. Nuptial coloration and mating success of male threespine stickleback breeding in a tide pool near Isle-Verte, Québec

Nuptial colour	Number of eggs	
	1991	1992
0	0 (0–1534, 15) <sup>a</sup>	0 (0–1526, 10) <sup>a</sup>
1	390 (0–1646, 36) <sup>a</sup>	0 (0–1902, 20) <sup>a</sup>
2	65 (0–1583, 32) <sup>a</sup>	81 (0–1960, 40) <sup>a</sup>
3	939 (0–2660, 11) <sup>b</sup>	0 (0–3717, 30) <sup>a</sup>

1991: Kruskal–Wallis ANOVA,  $H = 8.38$ , d.f. = 3,  $p = 0.04$ .

1992:  $H = 4.84$ , d.f. = 3,  $p = 0.18$ . Medians with same letter are not statistically different.

Data are medians (ranges and sample sizes (*N*)) of eggs in nests at the time of collection. Groups were compared with a Kruskal–Wallis ANOVA followed by a *post hoc* multiple comparison test.

number of parasites = 5 (0–20); males with eggs, 4.5 (0–24), ( $p = 0.29$ , Mann–Whitney *U* test)). There was no correlation between the Fulton index and the number of parasites (1991:  $r_s = -0.17$ ,  $p = 0.07$ ,  $n = 94$ ; 1992:  $r_s = -0.05$ ,  $p = 0.56$ ,  $n = 100$ ).

There was no association between Fulton's condition index and the degree of nuptial coloration (1991:  $r_s = -0.08$ ,  $p = 0.19$ ; 1992:  $r_s = 0.001$ ,  $p = 0.60$ ,  $n = 100$ ). There was a tendency for males with full development of the coloration (score of 3) to obtain more eggs than other categories of males (Table 2). A *post hoc* test revealed that these males received significantly more eggs than the other males, but that there were no differences in mating success among the first three groups. However, there was no evidence of such an association in 1992 (Table 2).

There was no association between parasite numbers and the degree of a male's nuptial coloration (1991:  $r_s = -0.01$ ,  $p = 0.54$ ,  $n = 94$ ; 1992:  $r_s = 0.06$ ,  $p = 0.87$ ,  $n = 100$ ).

#### Parental care and parasite load

Neither of our measures of parental care was associated with parasite load (aggression:  $r_s = 0.01$ ,  $p = 0.54$ ,  $n = 100$ ; return time:  $r_s = 0.10$ ,  $p = 0.32$ ,  $n = 100$ ).

*Parasites, female fecundity and physical condition*

The number (median = 5, range = 0–172,  $n = 270$ ) of parasites on these females did not differ from a comparable sample of males (median = 5, range = 0–28,  $n = 194$ ;  $p = 0.75$ , two-tailed Mann–Whitney  $U$  test). There were small but statistically significant negative correlations, between parasite load and physical condition index ( $K$ ) (Spearman's  $r_s = -0.14$ ,  $p = 0.03$ ,  $n = 270$ ) and between parasite load and clutch size ( $r_s = -0.12$ ,  $p = 0.04$ , 270) for female threespine sticklebacks of our population.

**Discussion**

The main goals of our study were to determine if 'blackspot' played any role in determining the outcome of male–male competition for territories and whether it affected female mate choice or male parental care in a field population of threespine sticklebacks. A second objective was to examine for possible effects on female clutch sizes and physical condition. Results from 2 years of field data and the laboratory fights are consistent and suggest that 'blackspot' has a minimal role, if any, on male stickleback behaviour of this population. As we found only a few fish (1 in 20) with any other parasites (nematodes), it seems safe to conclude that parasites have not played a major role in the evolution of the male's red coloration in this population.

Given our largely negative findings, it is reasonable to ask if there was any danger in committing a type 2 statistical error, that is, erroneously accepting the null hypothesis of no significant differences when in fact such differences occurred. We consider this unlikely for several reasons. First, our non-parametric tests are generally 90–95% as good as their non-parametric equivalents (Siegel and Castellan, 1988). The fact that we detected a small, albeit statistically significant, effect of 'blackspot' for our females may have occurred because of their greater reproductive investment. However, this was not measured and we feel that an experimental study infecting females with parasites should be done to verify whether the correlation is biologically meaningful.

With respect to the negative findings for the males, it is important to note that our sample sizes were as large or larger than those of other researchers who have reported effects of 'blackspot' disease on physical condition (e.g. Lemly, 1980). The literature concerning the effects of 'blackspot' disease is equivocal in that some authors found significant effects of the disease on health and growth (e.g. Lemly, 1980; Lemly and Esch, 1984) whereas others did not (e.g. Baker, 1985). It seems that much higher levels of infestation are needed to affect adult male sticklebacks than occurs at Isle-Verte. For example, Lemly and Esch (1984) reported that  $> 50$  cysts/fish were needed to detect effects on body condition in juvenile bluegill sunfish *Lepomis macrochirus*, whereas most of our adult fish, had less than 10 cysts.

Milinski and Bakker (1990) suggested that female threespine sticklebacks might use colour to discriminate among males because brightness (colour intensity) reveals to the females which males are in the best physical condition. Choosing bright males would be advantageous if these males were better able to care for her eggs than other males and/or if her sons inherited their father's 'parasite resistance' genes. Although our most colourful males obtained the most matings in 1 year of our study, their parasite loads did not differ from that of less colourful males.

The reason for the failure to find the same association between colour and mating success in the 2 years of the present study is unknown. In 1991 the breeding season was characterized mainly by bright sunny conditions whereas conditions in 1992 were often overcast. Perhaps, differences in light conditions were responsible for the observed differences in female choice. Our previous work at this site indicates that females use nest site characteristics, especially the amount of algal

cover surrounding the nest when choosing a spawning partner (FitzGerald, 1983). An experimental study to distinguish the relative importance of nest site characteristics and male characteristics, especially the red colour, is needed.

There was no evidence that parasite load had any effect on parental care. Heavily parasitized males invested as much in their broods as other males and they were able to successfully hatch their eggs (M. Fournier, personal observation). This finding suggests that females do not use variation in redness in choosing their mates because redder males are better parents.

To conclude, although the red colour may sometimes influence female choice in this system, the preference has probably not evolved through the Hamilton–Zuk mechanism.

## Acknowledgements

The Canadian Wildlife Service gave permission to work at the Isle-Verte Ecological Reserve. The study was supported by research grants from the National Science and Engineering Research Council of Canada (NSERC) and Fonds pour la Formation des Chercheurs et l'Aide à la Recherche (FCAR, Québec). Julie Morrisette held an FCAR postgraduate scholarship during the tenure of the study. Many people, especially Theo C. M. Bakker and Robert Poulin provided stimulating discussion on this topic. Jacques Bovet, Lee Dugatkin, Donald Kramer, Robert Poulin and Derek Roff all provided useful comments on several versions of the manuscript. However, this acknowledgement does not imply an unqualified blessing on its contents by anyone. Any remaining errors of fact or interpretation are our sole responsibility.

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