EFFECTS OF ALGAE COVER ON EGG ACQUISITION IN MALE THREE-SPINED STICKLEBACK

by

ULRIKA CANDOLIN[1,2]
(Department of Biological & Environmental Sciences, University of Helsinki)

(Acc. 15-III-2004)

Summary

The intensity of sexual selection depends on environmental conditions; factors such as predation risk and energy limitation determine the strength of mate choice and the intensity of sexual displays. Eutrophication of shallow coastal areas is changing the breeding habitats of the three-spined stickleback *Gasterosteus aculeatus* by increasing the growth of filamentous algae. Here I show that the density of breeding males is higher in habitats with a denser growth of filamentous algae, but the variation in egg number among nests is lower. This more equal distribution of eggs in densely vegetated habitats could be due to a lower variation among males in mating success, or to habitat-dependent frequencies of egg consumption, egg stealing or sneak fertilisation homogenising the distribution of eggs among nests in densely vegetated habitats. Of these possibilities, reduced variation among males in mating success is the most likely explanation, because increased vegetation reduces the opportunity for mate choice by reducing visibility and mate encounter rate. Variation in egg stealing and sneak fertilisation are less likely to explain the result because of their generally low frequency. Further experimental work is required to determine the causes and consequences of the habitat-dependent distribution of eggs among nests.

Introduction

Natural selection proceeds in interaction with the environment. The individuals that are best adapted to the environmental conditions pass most alleles

---

[1] Address: Department of Biological & Environmental Sciences, P.O. Box 65, FIN-00014, University of Helsinki, Finland; e-mail address: ulrika.candolin@helsinki.fi

[2] I thank Tiina Salesto for assistance in the field and the anonymous referees for helpful comments. The study was financed by the Academy of Finland, Oskar Öflunds Stiftelse and Svenska Kulturfonden.

on to the following generations (Darwin, 1859). Sexual selection is a subset of natural selection and similarly works in interaction with the environment. Factors such as predation risk, energy limitation and prevalence of parasites determine the strength of competition for mates and mate choice, the two components of sexual selection. For instance, mate choosiness decreases when the cost of choice increases due to increased predation risk or energy expenditure (Godin & Briggs, 1996; Jennions & Petrie, 1997; Luttbeg *et al.*, 2001), whereas the exaggeration of sexual displays decreases when the cost of displaying increases due to increased predation risk (Magnhagen, 1991; Candolin, 1997; Hedrick, 2000), higher prevalence of parasites (Milinski & Bakker, 1990; Zuk *et al.*, 1998), energy limitation (Møller & de Lope, 1994; Kotiaho, 2000), or increased socially imposed costs (Johnstone & Norris, 1993; Galeotti *et al.*, 1997; Candolin, 1999).

Human activities are currently altering habitats at a quicker pace than ever before. Little is known on how such activities will affect sexual selection, one of the major evolutionary forces. Eutrophication is a serious environmental problem throughout Europe, with notorious effects in the North Sea and the Baltic Sea (Bonsdorff *et al.*, 1997a, b). Eutrophication causes changes in the species composition of benthic algae, in particular by increasing the growth of filamentous algae at the expense of slow-growing macroalgae. This in turn alters the composition and dynamics of the littoral fauna community (Kraufvelin *et al.*, 2002; Bokn *et al.*, 2002; Bonsdorff *et al.*, 2002). However, the long-term evolutionary effects that such changes may have on ecosystems are wholly unknown.

The three-spined stickleback *Gaterosteus aculeatus* breeds in shallow coastal waters where males establish territories and build nests (Wootton, 1976). Males prefer to nest in vegetated areas concealed from predators and competing males (van den Assem, 1967; Jenni, 1972; Kraak *et al.*, 1999; Dzieweczynski & Rowland, 2004), which allows more vigorous courtship (Candolin & Voigt, 1998). However, increased vegetation may reduce mate encounter rate due to lower visibility in dense algae growth (Candolin & Voigt, 2001). Thus, habitat structure could influence both the intensity of sexual displays and mate encounters and thereby sexual selection. As a first step in investigating the effects of increased habitat structure on sexual selection, I studied the variation in the distribution of eggs among nests in habitats differing in the density filamentous algae. The variation in the number of eggs among nests could indicate the intensity of sexual selection, with larger deviation from random variation indicating higher intensity of selection (Andersson, 1994). This could arise from changes in the intensity of female choice or male-male competition. However, other factors than sexual selection could influence egg numbers, such as egg predation, and need to be considered when inferring the intensity of sexual selection from variation in egg numbers.

Method

Males nesting in habitats with different densities of algae were observed in a shallow bay near Tvärminne Zoological Station in the Baltic Sea in southern Finland (60°N, 23°E). Patches with different algae cover were selected and one male was arbitrarily selected from each patch by arbitrarily selecting a focal point within the patch and then selecting the closest territorial male as the focal male. Algae density was recorded by visually estimating the percentage of a 1 × 1 m area around the focal male that was covered by filamentous algae, mainly *Cladophora glomerata*. A scale from 0 up to 80% cover with 10% increments was used. Patches with different densities of algae were interspersed along the shore, at depths from 20-60 cm. In total, 45 males were sampled, 5 males from each of the nine categories of algae cover (0-80% cover).

The focal male was observed for 10 min and the size of his territory, the number of territorial males within the selected 1 × 1 m area, and the number of interactions with other sticklebacks, both males and females, were recorded. An interaction was defined as an approach by the male towards another stickleback that entered his territory, including approaches where the male did not swim all the way towards the intruder. Usually the intruding stickleback quickly left the territory when approached by the territorial male and only seldom was biting, chasing or courtship observed. The size of the territory was estimated as the area that the male defended against other sticklebacks. The boundaries were determined by observing the interactions of the male with neighbouring males and intruding sticklebacks; these boundaries were defined by the maximum distance from the nest at which the owner confronted and drove off would-be intruders. Territory size was approximated as length × width, since most territories stretched along large stones and were more rectangular than circular in form. Finally, the male was caught with a hand net and the tip of one of his dorsal spines was cut so that he could be recognised in the future. After marking, the male was released back into his territory. All males resumed normal territorial behaviours within 30 minutes.

Three to five days after first observing a territorial male, when the male had entered the parental phase, the male was caught with a hand net and measured for standard length. To determine mating success, the male's nest was collected and brought to the laboratory where the number of eggs were counted. For males that had disappeared since the first observation, mating success was set to zero.

The density of territorial males and the frequency of interactions with other fish were square root + 0.5 transformed before analyses. To test whether the measured variables changed with algae density, linear and quadratic regression was carried out. To test whether the variation among males in the measured traits changed with algae density, coefficients of variation, CV (SD/mean), were calculated for each algae density and changes in these coefficients were tested using linear regression.

Results

The density of territorial males increased with the density of filamentous algae in the habitat (Tables 1 and 2, Fig. 1). At the same time the number of approaches by the territorial fish towards bypassing sticklebacks (interactions) decreased (Tables 1 and 2). The size of both the males and their territories showed a tendency to be larger at intermediate algae densities, but this was not statistically significant (Tables 1 and 2). The number of eggs in the nests did not change with algae density (Tables 1 and 2, Fig. 1).

Although the number of eggs in the nests did not change, the variation in the number of eggs among nests decreased with increasing algae density

TABLE 1. *Means ± SD for the measured variables of males nesting at different algae densities*

Algae cover (%)	Nr of territories/ m^2	Body length, mm (SL)	Territory size, m^2	Interactions/ 10 min	Nr of eggs in nest
0	0.40 ± 0.55	51.0 ± 2.4	0.13 ± 0.03	6.40 ± 4.16	191 ± 141
10	0.80 ± 0.45	51.8 ± 1.9	0.22 ± 0.13	8.80 ± 4.82	205 ± 177
20	0.60 ± 0.55	52.2 ± 3.7	0.23 ± 0.12	5.40 ± 3.98	256 ± 209
30	0.60 ± 0.89	51.2 ± 3.3	0.22 ± 0.16	8.60 ± 3.98	181 ± 133
40	1.00 ± 1.00	53.0 ± 4.5	0.25 ± 0.09	6.00 ± 4.90	291 ± 241
50	2.00 ± 1.00	56.2 ± 5.3	0.28 ± 0.12	4.60 ± 2.70	303 ± 171
60	2.00 ± 1.22	55.4 ± 2.7	0.30 ± 0.10	2.40 ± 1.14	186 ± 84
70	2.80 ± 1.10	53.0 ± 1.9	0.23 ± 0.10	5.20 ± 1.92	217 ± 75
80	2.60 ± 0.89	51.4 ± 3.5	0.21 ± 0.13	0.80 ± 0.84	158 ± 79

Five males were sampled for each algae cover category.

TABLE 2. *The dependence of male and territory traits on algae density*

	r^2	$F_{1,43 \text{ or } 1,42*}$	p	Slope
Territory density	0.46	38.90	<0.001[†]	b1 = 0.012
Body length (SL)	0.11	2.60*	0.086	b2 = −0.002
Territory size	0.13	3.09*	0.056	b2 = −0.006
Interactions with other fish	0.25	14.56	<0.001[†]	b1 = −0.02
Nr of eggs in nest	0.00	0.05	0.821	

Linear and *quadratic regression was used for analyses.

[†] Significance remains after a table-wise sequential Bonferroni correction.

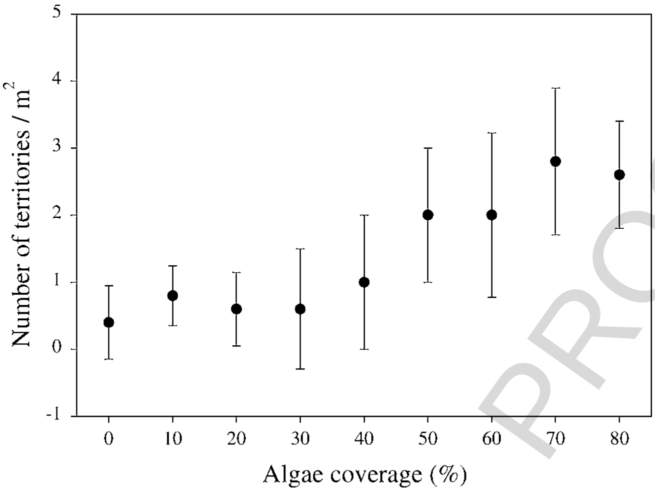

Fig. 1. Mean ± SD density of territories around a focal male at different algae densities. Five males were sampled for each algae cover category.

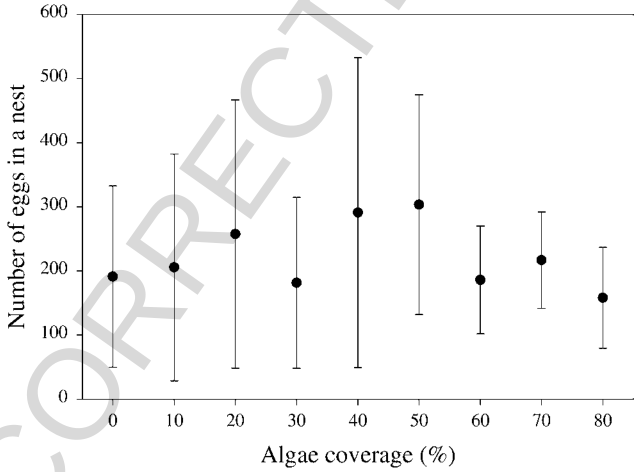

Fig. 2. Mean ± SD number of eggs in nests at different algae densities. Five males were sampled for each algae cover category.

(Table 3, Fig. 2). No significant changes in variance were detected for the other measured traits; the size of the male and his territory, territory density and frequency of interactions with bypassing sticklebacks (Table 3).

TABLE 3. *The dependence of the variation (CV) in male and territory traits on algae density*

	r^2	$F_{1,7}$	p	Slope
Territory density	0.29	2.86	0.135	
Body length (SL)	0.02	0.13	0.727	
Territory size	0.02	0.11	0.752	
Interactions with other fish	0.05	0.38	0.559	
Nr of eggs in nest	0.69	15.34	$0.006^{\dagger}$	$b1 = -0.006$

Regression was used for analyses.

† Significance remains after a table-wise sequential Bonferroni correction.

Discussion

The density of territorial males was higher in habitats with a denser growth of filamentous algae. However, the variation in the number of eggs among nests decreased with increased algae growth. This more equal distribution of eggs in more vegetated habitats could be due to (1) habitat-dependent egg mortality through predation or cannibalism, (2) habitat-dependent frequency of egg stealing and sneak fertilisation, or (3) less variation in mating success among males in densely vegetated habitats. The two latter possibilities imply that changes in vegetation structure could bring with it changes in sexual selection, through changes in the intensity or outcome of female mate choice and male-male competition, such as through changes in egg cannibalism, sneak fertilisations, courtship interruptions or courtship activity.

Habitat-dependent egg mortality through predation or cannibalism could result in a more equal distribution of eggs in densely, as opposed to sparsely, vegetated habitats, if the variation in egg number among nests is reduced in densely vegetated habitats or increased in sparsely vegetated habitats. A few studies have found egg predation and cannibalism to depend on vegetation cover, with exposed nests being more susceptible (Sargent & Gebler, 1980; Whoriskey & FitzGerald, 1985; Mori, 1993). However, whether a higher rate of egg disappearance in exposed areas results in increased variation among males in egg numbers is unknown. Filial cannibalism, with the father eating his own eggs, is a major source of egg disappearance in sticklebacks (FitzGerald, 1991; Candolin, 2000). It is expected when the cost of caring for eggs exceeds the benefit (Sargent, 1992), such as under poor food or oxygen conditions (Lindström, 1998; Payne *et al.*, 2002; Lissåker *et al.*, 2003).

Whether the incidence of filial cannibalism varies with habitat structure is, however, unknown. More studies are needed to determine whether habitat-dependent egg cannibalism and predation could contribute to the habitat-dependent distribution of eggs among nests.

Habitat-dependent frequency of egg stealing and sneak fertilisation are unlikely to have had a large effect on the distribution of eggs among nests. Both behaviours are generally infrequent (Goldschmidt & Bakker, 1990; Rico *et al.*, 1992; Foster, 1994; Largiader *et al.*, 2001), although the incidence in the present population remains to be determined. Whether the frequency of egg stealing and sneak fertilisations varies with the density of vegetation and contributed to the difference in the distribution of eggs is nevertheless worth investigating.

The last hypothesis, that the reduced variation in egg numbers was due to reduced variation among males in mating success could be true if increased vegetation reduced male-male competition (intra-sexual selection) or female mate choice (inter-sexual selection). Earlier studies by Sargent & Gebler (1980) and Sargent (1982) show that courtship intrusions by neighbouring males are more frequent in open habitats and influence female mate choice. Thus, increased vegetation could allow less attractive or less competitive males to mate because of less distractions from other competing males during courtship, which would relax the strength sexual selection (Sargent, 1982; Ward & FitzGerald, 1987; Candolin, 1999; Dzieweczynski & Rowland, 2004).

The other scenario, of reduced variation in mating success due to reduced female mate choice in heavily vegetated habitats, could arise if the choosiness of the females, or the variation among males in the phenotypic traits used as cues in female mate choice, are reduced in more vegetated areas. Some support for reduced female mate choosiness in heavily vegetated habitats has been gained in an earlier study on the same population where the encounter rate with females increased when the structural complexity of a territory was reduced by removing stones overgrown by algae (Candolin & Voigt, 2001). This enhanced encounter rate in open areas might have arisen from improved visibility increasing the number of males that a female detected within a given time span. Thus, increased vegetation could reduce encounter rate with males and the opportunity of mate choice. On the other hand, the increase in the density of territorial males with algae density could counteract this effect by increasing encounter rates. Interestingly, the number of interactions with other fish decreased with algae density, suggesting a reduction in mate encounter rate with increased vegetation.

The habitat-dependent distribution of eggs appears less likely to be the result of habitat-dependent changes in the variation among males in phenotypic traits used as cues in female mate choice, since the variation among males in body size or territory size did not depend on habitat structure. A possibility remains, however, that variation in traits not investigated in this study, such as red nuptial coloration or courtship activity, changed with habitat structure. This is possible as a few studies have shown that males adjust their courtship activity to concealment from predators and rivalling males (Candolin & Voigt, 1998; Dzieweczynski & Rowland, 2004).

Support for an effect of the habitat on sexual selection has also been gained in a study on another littoral nest-spawning fish, the sand *goby Pomatoschistus minutus*, where the potential for intra-sexual selection increased when the availability of nests for males was low, whereas the potential for inter-sexual selection increased when nests were common (Forsgren *et al.*, 1996). What long-term effects changes in the intensity of intra- and intersexual selection will have, for gobies, sticklebacks and other species, are unknown. Surprisingly little attention has been paid to the consequence that habitat changes may have on sexual selection. Research on cichlids fishes of Lake Victoria shows that eutrophication and increased turbidity of water interferes with mate choice and relaxes sexual selection, which in turn influences speciation (Seehausen *et al.*, 1997). The growth of filamentous algae is currently increasing along the coast of the Baltic Sea (Bonsdorff *et al.*, 2002), changing the breeding habitats of the stickleback. This study shows that this can result in a more even distribution of eggs, possible through a reduction in the intensity of sexual selection. Whether this will influence the further evolution of the stickleback would deserve more attention.

References

Andersson, M. (1994). Sexual selection. — Princeton University Press, Princeton.

Assem, J. van den (1967). Territoriality in the three-spined stickleback *Gasterosteus aculeatus* L. An experimental study in intraspecific competition. — Behaviour Suppl. 16, p. 1-164.

Bokn, T.L., Moy, F.E., Christie, H., Engelbert, S., Karez, R., Kersting, K., Kraufvelin, P., Lindblad, C., Marba, N., Pedersen, M.F. & Sorensen, K. (2002). Are rocky shore ecosystems affected by nutrient-enriched seawater? Some preliminary results from a mesocosm experiment. — Hydrobiol. 484, p. 167-175.

Bonsdorff, E., Blomqvist, E.M., Mattila, J. & Norkko, A. (1997a). Coastal eutrophication: Causes, consequences and perspectives in the Archipelago areas of the northern Baltic Sea. — Estuar. Coast. Shelf Sci. 44, p. 63-72.

— —, — —, — — & — — (1997b). Long-term changes and coastal eutrophication. Examples from the Aland Islands and the Archipelago Sea, northern Baltic Sea. — Oceanol. Acta 20, p. 319-329.

— —, Rönnberg, C. & Aarnio, K. (2002). Some ecological properties in relation to eutrophication in the Baltic Sea. — Hydrobiol. 475, p. 371-377.

Candolin, U. (1997). Predation risk affects courtship and attractiveness of competing threespine stickleback males. — Behav. Ecol. Sociobiol. 41, p. 81-87.

— — (1999). Male-male competition facilitates female choice in sticklebacks. — Proc. R. Soc. Lond. B 266, p. 785-789.

— — (2000). Changes in expression and honesty of sexual signalling over the reproductive lifetime of sticklebacks. — Proc. R. Soc. Lond. B 267, p. 2425-2430.

— — & Voigt, H.-R. (1998). Predator-induced nest site preference: safe nests allow courtship in sticklebacks. — Anim. Behav. 56, p. 1205-1211.

— — & — — (2001). Correlation between male size and territory quality: consequence of male competition or predation risk? — Oikos 95, p. 225-230.

Darwin, C. (1859). On the origin of species by means of natural selection. — Murray, London.

Dzieweczynski, T.L. & Rowland, W.J. (2004). Behind closed doors: use of visual cover by courting male threespine stickleback, *Gasterosteus aculeatus*. — Anim. Behav. (in press).

FitzGerald, G.J. (1991). The role of cannibalism in the reproductive ecology of the threespine stickleback. — Ethology 89, p. 177-194.

Forsgren, E., Kvarnemo, C. & Lindström, K. (1996). Mode of sexual selection determined by resource abundance in two sand goby populations. — Evolution 50, p. 646-654.

Foster, S.A. (1994). Evolution of the reproductive behaviour of threespine stickleback. — In: The evolutionary biology of the threespine stickleback (M.A. Bell & S.A. Foster, eds). Oxford University Press, Oxford, p. 381-398.

Galeotti, P., Saino, N., Sacchi, R. & Møller, A.P. (1997). Song correlates with social context, testosterone and body condition in male barn swallows. — Anim. Behav. 53, p. 687-700.

Godin, J.G.J. & Briggs, S.E. (1996). Female mate choice under predation risk in the guppy. — Anim. Behav. 51, p. 117-130.

Goldschmidt, T. & Bakker, T.C.M. (1990). Determinants of reproductive success of male sticklebacks in the field and in the laboratory. — Neth. J. Zool. 40, p. 664-687.

Hedrick, A.V. (2000). Crickets with extravagant mating songs compensate for predation risk with extra caution. — Proc. R. Soc. Lond. B 267, p. 671-675.

Jenni, D.A. (1971). Effects of conspecifics and vegetation on nest site selection in *Gasterosteus aculeatus* L. — Behaviour 42, p. 97-118.

Jennions, M.D. & Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. — Biol. Rev. 72, p. 283-327.

Johnstone, R.A. & Norris, K. (1993). Badges of status and the cost of aggression. — Behav. Ecol. Sociobiol. 32, p. 127-134.

Kotiaho, J.S. (2000). Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. — Behav. Ecol. Sociobiol. 48, p. 188-194.

Kraak, S.B.M., Bakker, T.C.M. & Mundwiler, B. (1999). Sexual selection in sticklebacks in the field: correlates of reproductive, mating, and paternal success. — Behav. Ecol. 10, p. 696-706.

Kraufvelin, P., Christie, H. & Olsen, M. (2002). Littoral macrofauna (secondary) responses to experimental nutrient addition to rocky shore mesocosms and a coastal lagoon. — Hydrobiol. 484, p. 149-166.

Largiader, C.R., Fries, V. & Bakker, T.C.M. (2001). Genetic analysis of sneaking and egg-thievery in a natural population of the three-spined stickleback (*Gasterosteus aculeatus* L.). — Heredity 86, p. 459-468.

Lindström, K. (1998). Effects of costs and benefits of brood care on filial cannibalism in the sand goby. — Behav. Ecol. Sociobiol. 42, p. 101-106.

Lissåker, M., Kvarnemo, C. & Svensson, O. (2003). Effects of a low oxygen environment on parental effort and filial cannibalism in the male sand goby, *Pomatoschistus minutus*. — Behav. Ecol. 14, p. 374-381.

Luttbeg, B., Towner, M.C., Wandesforde-Smith, A., Mangel, M. & Foster, S.A. (2001). State-dependent mate-assessment and mate-selection behavior in female threespine sticklebacks (*Gasterosteus aculeatus*, Gasterosteiformes: Gasterosteidae). — Ethology 107, p. 545-558.

Magnhagen, C. (1991). Predation risk as a cost of reproduction. — Trends Ecol. Evol. 6, p. 183-186.

Milinski, M. & Bakker, T.C.M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. — Nature 344, p. 330-333.

Møller, A.P. & De Lope, F. (1994). Differential costs of a secondary sexual character: An experimental test of the handicap principle. — Evolution 48, p. 1676-1683.

Mori, S. (1993). The breeding system of the three-spined stickleback, *Gasterosteus auleatus* (*forma leiura*) with reference to spatial and temporal patterns of nesting acitivity. — Behaviour 126, p. 97-124.

Payne, A.G., Smith, C. & Campbell, A. (2002). Filial cannibalism improves survival and development of beaugregory damselfish embryos. — Proc. R. Soc. Lond. B 269, p. 2095-2102.

Rico, C., Kuhnlein, U. & FitzGerald, G.J. (1992). Male reproductive tactics in the threespine stickleback: an evaluation by DNA fingerprinting. — Mol. Ecol. 1, p. 79-87.

Sargent, R.C. (1982). Territory quality, male quality, courtship intrusions, and female nest-choice in the threespine stickleback, *Gasterosteus aculeatus*. — Anim. Behav. 30, p. 364-374.

— — (1992). Ecology of filial cannibalism in fish: theoretical perspectives. — In: Cannibalism: ecology and evolution among diverse taxa (M.A. Elger & B.J. Crespi, eds). Oxford University Press, Oxford, p. 38-62.

— — & Gebler, J.B. (1980). Effects of nest site concealment on hatching success, reproductive success, and paternal behaviour of the threespine stickleback, *Gasterosteus aculeatus*. — Behav. Ecol. Sociobiol. 7, p. 137-142.

Seehausen, O., Alphen, J.J.M. & Witte, F. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. — Science 277, p. 1808-1811.

Ward, G. & FitzGerald, G.J. (1987). Male aggression and female mate choice in the threespine stickleback, *Gasterosteus aculeatus* L. — J. Fish Biol. 30, p. 679-690.

Whoriskey, F.G. & FitzGerald, G.J. (1985). Nest sites of the threespine stickleback: can site characteristics alone protect the nest sites against egg predators and are nest sites a limiting resource. — Can J. Zool. 63, p. 1991-1994.

Wootton, R.J. (1976). The biology of the sticklebacks. — Academic Press, London.

Zuk, M., Rotenberry, J.T. & Simmons, L.W. (1998). Calling songs of field crickets (*Teleogryllus oceanicus*) with and without phonotactic parasitoid infection. — Evolution 52, p. 166-171.