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ORIGINAL ARTICLE

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Effects of increased vegetation cover on nesting behavior of sticklebacks (*Gasterosteus aculeatus*)

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Abstract To maximize reproductive success, reproductive behaviors have to be adjusted to environmental conditions. The breeding habitats of the threespine stickleback *Gasterosteus aculeatus* are presently changing in the Baltic Sea due to eutrophication and enhanced growth of filamentous green algae. Earlier studies show that high algae density reduces mate encounter rate and the intensity of selection on a few sexually selected traits, but the willingness of males to nest under the new conditions, and their investment into reproduction, have not been investigated. Here, we investigated the effect that increased algae growth has on nesting behavior of sticklebacks. We allowed males to build nests in aquaria with either a sparse or a dense vegetation structure and recorded male nest building behavior and nest characters. Males that nested in dense vegetation showed a longer latency before commencing nest building and also completed nest building later since there was no difference in time spent building. There was no effect of vegetation cover on nest characters. These results suggest that males prefer to nest in sparser vegetation, but that they eventually nest in dense vegetation if more open habitats are not available. This may arise from a negative effect of vegetation on visibility and mate encounter rate and thus on expected reproductive success. This implies that human-induced changes in the environment may force sticklebacks to breed in non-preferred habitats. Since the intensity of sexual selection is reduced in these areas, this could influence the further evolution of the population.

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Introduction

Reproductive behaviors are, over evolutionary time, adjusted to environmental conditions to maximize reproductive success. Traits such as courtship activity, mate choice, mating behavior and parental effort are adjusted to factors like predation risk (Moodie 1972; Endler 1987; Lima and Dill 1990; Travers and Sih 1991; Resetarits and Wilbur 1991), resource availability and population demography (Rodd and Sokolowski 1995; Clutton-Brock et al. 1997; Krupa and Sih 1993; Kvarnemo and Ahnesjö 1996; Evans and Magurran 1999; Jirotkul 1999).

Due to human activities, the earth's habitats are currently changing more rapidly than ever before. Intensive agriculture, forest management, urbanization and pollution are disrupting nature and natural landscapes. The consequences this may have for evolutionary processes, and especially sexual selection, are little known. Sudden changes in the environment could affect who reproduces and influence the selection on traits and the evolution of populations. In support of this, a few studies find the intensity and direction of sexual selection to vary among populations depending on environmental conditions (Houde and Endler 1990; Arnqvist 1992; Endler and Houde 1995; Seehausen et al. 1997; Hill et al. 1999; Simmons et al. 2001; Kwiatkowski and Sullivan 2002).

The environment of the Baltic Sea is presently changing due to eutrophication. Increased input of nutrients is enhancing the growth of filamentous algae and phytoplankton, resulting in changed habitat structure and turbid water (Bonsdorff et al. 2002; Bokn et al. 2002). In particular, the growth of filamentous algae like *Cladophora* and *Pilayella* is increasing at the expense of slow-growing macro algae like *Fucus vesiculosus*. A species that spawns in this changing environment is the threespine stickleback, *Gasterosteus aculeatus*. Males establish territories in shallow water and build nests out of algae. They court females for spawnings and then care alone for the eggs and the newly hatched fry for 2 to 3 weeks (Wootton 1976).

Several studies demonstrate that the reproductive behaviors of sticklebacks are adjusted to environmental conditions. Predation risk, for instance, influences the intensity of courtship activity (Candolin 1997) and nuptial coloration (Candolin 1998) and the choice of nest site (Candolin and Voigt 1998). Habitat structure and vegetation cover, on the other hand, determine the size of the territory and courtship activity, with territories being smaller in more vegetated habitats (Candolin and Voigt 2001a) and courtship activity depending on concealment from predators (Candolin and Voigt 1998) and competing males (Dzieweczynski and Rowland 2004). However, whether changes in the environment influence nest-building activities is unknown.

The stickleback male builds the nest by gluing together filamentous algae with Spiggin, a glycoprotein that is produced by the kidney and under the control of androgenic hormones (Jakobsson et al. 1999; Barber et al. 2001). The nest takes the form of a few-centimeters-long tunnel through which the female and the male can pass during spawning (Wootton 1976). The nest may be built on sand, on stones or in crevices between or under stones. Some degree of algae and macrophyte cover is beneficial since it correlates positively with the physiological status, size and redness of the male and with the number of eggs in the nests and with the hatching success (Guderley and Guevara 1998; Kraak et al. 1999, 2000). This is probably due to the advantage of vegetation cover in concealing the nest against predators and competitors. However, dense vegetation cover also reduces visibility and mate encounter rate (Candolin and Voigt 2001a), which implies that males have to trade between the costs and benefits of nesting in dense algae when choosing a nest site. Moreover, the intensity of sexual selection on red nuptial coloration and courtship behavior is reduced in habitats with dense growth of algae (Candolin 2004; Candolin, Salesto and Evers, unpublished data), which suggests that males are not adapted to the habitat.

Here, the aim is to investigate the effects that increased growth of filamentous green algae has on nest-building activities of sticklebacks. The nest is of crucial importance for successful reproduction since it both serves as a cue in female mate choice (Sargent 1982) and protects the eggs and the newly hatched fry against predators, both con- and heterospecific predators (Sargent and Gebler 1980). Changes in nest-building behavior could therefore have significant effects on reproductive success and sexual selection.

Methods

Sticklebacks were caught with Plexiglas traps (Candolin and Voigt 2001b) from Vindskär Bay close to Tvärminne Zoological station (60°N, 23°E) at the beginning of May before the breeding season. The fish were housed in flow-through aquaria under natural light and water temperature conditions for 1–3 weeks. They were fed daily on frozen chironomid larvae. Fish that turned out to be males, as determined by the development of blue eye color, were transferred to separate holding aquaria. A lack of suitable nesting materials discouraged breeding behavior in the holding tanks.

To determine the effect of algal cover on nest-building activities, 40 randomly chosen males were transferred to individual aquaria (60×30×30 cm) that differed in algal cover, i.e. sparse or dense vegetation cover. The males were measured for standard length to the nearest millimeter and weighed to the nearest 0.01 g before being placed in the aquaria. Half of the males (20 males) experienced a sparsely vegetated habitat containing only a nesting dish at one of the ends of the aquarium. The nesting dish (14 cm in diameter) was filled with 0.5 dl of sand, 20 g of live filamentous green algae (*Cladophora clomerata*) for nest construction and artificial algae made out of a bunch of 15-cm-long strings (see Candolin 1997). The rest of the males experienced a densely vegetated habitat with four additional bunches of artificial algae distributed over the bottom so that about 75% of the bottom, up to a height of 15 cm, was covered by strings of artificial algae. The artificial algae mimicked filamentous algae, and the density represented the density of algae found in nature, with the treatment with dense vegetation corresponding to a very high density of algae in the field.

The males were observed at least four times a day, and the number of days it took until a male started nest building and until he had constructed a complete nest and performed the creeping-through behavior, which indicates that the nest is ready (van Iersel 1953), was counted. Females were not presented to the males during this time. Males that did not complete nest building within 14 days were excluded from the analyses (two males in the sparse vegetation treatment, one male in the dense vegetation treatment) and replaced with new males. To determine nest characteristics, the completed nest was gently removed from the nesting dish and photographed from above with a digital camera against a white background containing a ruler for calibration of area. The total area and the "density" of the nest were measured from the digital images using ImageJ v.1.28u (http://rsb.info.nih.gov/ij/). "Density" or compactness of the nest was measured by transforming the images to a grey scale and measuring the mean grey of the selected nest area. The grey value approximated the density of the nest since the white background showed through depending on the thickness of the nest. To determine the total amount of material used for nest construction, the nest was dried to constant weight at room temperature and weighed to the nearest 0.001 g.

To analyze for effects of algae cover on the time until nest building, non-parametric Mann–Whitney U test was used since the data could not be transformed to normality. Data on nest characters fulfilled the requirements of parametric tests, and t tests were used to test for effects of habitat on nest characters. To analyze for correlations between the time until a male had built a nest and nest characters, Spearman rank correlation was used, due to the

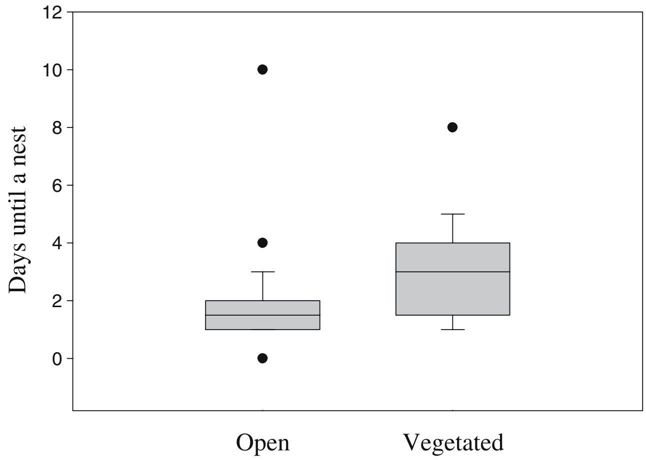

Fig 1 Days until males completed nest construction in open and vegetated habitats. The median, 10th, 25th, 75th and 90th percentiles and outliers are shown

non-normal distribution of the time data. To determine whether nest characters depended on male size and whether the relationship varied between habitats, an analysis of covariance was carried out. Correlations between male size and time until nest completion were tested using Spearman rank correlation.

Results

Males nesting in dense vegetation both started (Mann–Whitney *U* test; U=109, $N1$=$N2$=20, P=0.008) and completed (U=113, P=0.015, Fig. 1) nest construction later than males nesting in sparse vegetation. The time spent building the nest did not depend on vegetation cover (U=170, P=0.29). Since the latency until commencing nest building and the time until nest completion were strongly correlated for both treatments (sparse vegetation, r=0.97, P<0.001; dense vegetation, r=0.98, P<0.001), only the results for the number of days until nest completion are presented in the following.

Vegetation cover had no effect on the area or the density of the nests or on the amount of algae and sand that was used for nest building (Table 1). No significant correlations were found between the time until nest completion and nest characters such as nest area (Spearman rank correlation, N=40, r=−0.05, P=0.74), nest density (r=0.02, P=0.90) or nest dry weight (r=0.01, P=0.98). Qualitatively similar results were gained when the analyses are carried out for the two habitats separately (all p>0.1).

Table 1 Characters of nests built in open and vegetated habitats

Nest character	Open (N=20)	Vegetated (N=20)		
	Mean±SE	Mean±SE	t	P
Nest dry weight (g)	1.68±0.09	1.72±0.09	0.29	0.77
Nest area (cm^2)	24.86±0.75	25.81±1.15	0.70	0.49
Nest density	61.89±2.22	60.62±2.08	0.42	0.68

Table 2 Dependence of nest characters on male size and vegetation

	Male length		Vegetation		Length × vegetation	
	F	P	F	P	F	P
Nest dry weight (g)	4.26	0.046	0.98	0.328	1.02	0.318
Nest area (cm^2)	2.22	0.145	1.36	0.250	1.47	0.234
Nest density	0.66	0.420	1.45	0.236	1.40	0.244

N=40

Larger males built heavier nests, independent of habitat structure, but there was no significant effect of male size on the other measured nest characters (Table 2). The non-significant main effects remained when the non-significant interaction terms were removed from the models. Male size had no significant effect on the time it took until a male had built a nest when the two habitats were pooled (r=−0.03, P=0.83) or when the analysis was carried out for the two habitats separately (sparse vegetation, r=−0.22, P=0.34; dense vegetation, r=0.09, P=0.70).

Discussion

Dense algal cover increased the latency until commencing nest building and the time before the male possessed a complete nest, but had no significant effects on the time a male spent building a nest or on nest characters, such as the size or the density of the nests or on the amount of algae and sand that was used for nest construction. The delay in nest construction suggests that males were less motivated to build nests in dense vegetation, whereas the lack of an effect on nest characters indicate that the effort males put on nest building, after deciding to nest, did not depend on habitat quality.

The delay in nest building may be due to males experiencing the habitat as less ideal for mate attraction and reproduction. An earlier field study found the encounter rate with females to decrease when algae cover increased, probably due to reduced visibility in dense vegetation (Candolin and Voigt 2001a). In addition, a laboratory study showed that the cost of courtship is higher in dense vegetation, in terms of increased time spent courting and increased courtship intensity, which may similarly arise from reduced visibility in dense vegetation and impaired ability of females to evaluate males (Candolin, Salesto and Evers, unpublished data). Thus, nesting in dense vegetation may be costly in terms of reduced mate encounter rate and increased required investment in courtship.

Increased algae cover could, however, also be favorable in protecting the male and the eggs and fry against predators (Sargent and Gebler 1980). In support of this, moderately increased algae cover has been shown to allow more courtship in the presence of predators (Candolin and Voigt 1998). Moreover, males that nest close to vegetation have a higher hatching success than males nesting in the open (Kraak et al. 1999), although this correlation could arise from better fathers choosing vegetated nest sites and not from vegetation cover improving hatching success. Thus, increased vegetation could have both positive and negative effects on reproduction, forcing males to trade between the benefits and costs when choosing a nest site. It is likely that moderately vegetated areas that ensure a high female encounter rate while still protecting the male and the eggs against predators are most favorable for reproduction. The vegetation cover in this study might have been too dense to be favorable for sticklebacks. In the field, the highest density of sticklebacks is usually found in moderately vegetated habitats, where the vegetation density is lower than in the high-density treatment (Candolin, personal observation). In these areas, enough algae are growing to provide protection against predators while not profoundly decreasing visibility.

Effects of algae cover on reproductive traits, such as nest construction (this study) and courtship behavior (Candolin, Salesto and Evers, unpublished data) can have further consequences for sexual selection. Males of high quality, who would be expected to prefer to nest in moderately vegetated habitats, may be forced to spawn in densely vegetated habitats when sparsely vegetated habitats decrease in abundance due to eutrophication. This could decrease their reproductive success compared to more sparsely vegetated habitats and influence sexual selection. Support for an effect of algae cover on the intensity of sexual selection has been gained in a correlative field study where the variation among males in the number of eggs in their nests was lower in densely vegetated habitats than in sparsely vegetated habitats (Candolin 2004), and in a laboratory study where increased vegetation cover reduced the intensity of sexual selection on different male visual traits (Candolin, Salesto and Evers, unpublished data).

Studies on a variety of organisms show that the intensity and direction of sexual selection vary among populations depending on environmental conditions (Arnqvist 1992; Forsgren et al. 1996; Boughman 2001; Sih et al. 2002; Lehtonen and Lindström 2004). However, the effect of human-induced changes in the environment on sexual selection has received less interest (Seehausen et al. 1997; Järvenpää and Lindström 2004). Sticklebacks are evolutionary adaptive and have adapted to a range of new environments (Schluter and McPhail 1992; Taylor and McPhail 2000; Boughman 2001; McPhail 1994; McKinnon and Rundle 2002; Bell et al. 2004; Doucette et al. 2004; McKinnon et al. 2004). The consequences that the growth of filamentous algae may have for the viability and further evolution of the stickleback populations in the Baltic Sea are unknown, but would deserve more attention.

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