Evolutionary Ecology Research, 2011, **13**: 697–710

Adaptive phenotypic differentiation of courtship in response to recent anthropogenic disturbance

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ABSTRACT

Background: Anthropogenic activities are causing ecological changes, including eutrophication, which can induce behavioural alterations in animals. Male threespine stickleback, *Gasterosteus aculeatus*, need to increase their courtship intensity to maintain a high mating success in eutrophied habitats.

Question: Has the courtship behaviour of threespine stickleback populations differentiated in response to spatial variation in human-induced eutrophication?

Populations: Parallel pairs of threespine stickleback populations in the Baltic Sea that have been exposed to mild and severe human-induced eutrophication during the last decades.

Methods: Males from mildly and severely eutrophied habitats were allowed to court dummy and live females under standardized conditions. We measured the frequency of different courtship behaviours.

Results: Males from severely eutrophied habitats courted more intensively.

Conclusions: Stickleback populations have differentiated phenotypically in courtship behaviour in response to spatial variation in human-induced eutrophication. The differentiation should improve individual fitness.

Keywords: environmental change, mate choice, parallel divergence, phenotypic plasticity, sexual selection.

INTRODUCTION

Organisms can survive changing conditions in three main ways: they can disperse, adjust through phenotypic plasticity, or adapt through genetic changes. Currently, human activities are altering habitats at an accelerating rate. The first response of animals to these changes is usually behavioural, with the response depending on the animals' genetically determined behavioural reaction norms and on changes to these norms over their lifetime, through, for instance, learning (West-Eberhard, 2003; Sih *et al.*, 2011; Tuomainen and Candolin, 2011). Over time, genetic changes may gradually accumulate and result in the evolutionary adaptation of the population to the new conditions, depending on evolutionary constraints (Futuyma, 2010).

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Behavioural responses that alleviate negative impacts of the changing conditions on individual fitness can enhance population persistence. Optimal behavioural responses can maintain positive population growth, while beneficial but not optimal responses can reduce fitness losses and prevent population declines. This can give additional time for genetic adaptation and expose new phenotypes to selection (Pigliucci, 2001; Ghalambor *et al.*, 2007; Lande, 2009; Chevin and Lande, 2010). Maladaptive behavioural responses, on the other hand, such as those triggered by evolutionary traps (Schlaepfer *et al.*, 2002), can drive populations into decline.

The influence of anthropogenic activities on the environment usually varies spatially. This forces animals to adjust their behaviour to local conditions to maintain a high fitness, through phenotypic plasticity and/or genetic adaptation. This can in turn result in the phenotypic differentiation of populations (e.g. Reznick *et al.*, 1997; Grant, 1999; Stockwell *et al.*, 2003; Hendry *et al.*, 2008; Harris *et al.*, 2011; Westley, 2011). Correspondingly, separate populations that confront similar changes in their habitat can diverge in parallel in their traits (Losos, 2011; Wake *et al.*, 2011). Guppies, *Poecilia reticulata*, for instance, have diverged in life-history patterns in relation to predation risk in parallel series of locations on the slopes of the Northern Range Mountains of Trinidad (Reznick *et al.*, 1996), while *Anolis* lizards have evolved similar ecomorphs in response to similar selective pressures on islands among the Greater Antilles (Losos, 2009).

Few studies have, however, investigated parallel (or convergent) divergence of populations in relation to rapid human-induced environmental transitions. Thus, an important and largely unanswered question is whether species are able to adjust predictably to human disturbances. Sexually selected traits are ideal candidates for investigating parallel divergence, as the traits are sensitive to changes in the environment. The production, transmission, and reception of the information content of the traits depend on the environment, and animals consequently have to adjust these traits to suit changes in local conditions (Andersson, 1994; Candolin and Heuschele 2008). A failure to adjust can cause population declines and hybridizations between closely related species (Seehausen *et al.*, 1997; Lamont *et al.*, 2003; Taylor *et al.*, 2006; Mercader *et al.*, 2009; Gilman and Behm, 2011; Volanthen *et al.*, 2012).

Currently, we have little information on the degree to which populations can differentiate adaptively in sexually selected traits in response to rapid human-induced changes (Candolin and Wong, 2012). A growing number of studies have found that species plastically alter their sexually selected behaviour in response to rapidly changing conditions (e.g. Candolin *et al.*, 2007; Lengagne, 2008; Heuschele *et al.*, 2009; Verzijden *et al.*, 2010), but whether the responses are beneficial or not is seldom known (Candolin and Heuschele 2008; van der Sluijs *et al.*, 2011). Moreover, there are few documented cases of contemporary evolution of secondary sexual traits, compared with the evidence available for naturally selected traits (Svensson and Gosden, 2007). Parallel differentiation of sexually selected traits in response to similar changes in the environment because of human activities would indicate the ability of species to adjust predictably to these changes.

We investigated if populations of threespine stickleback, *Gasterosteus aculeatus*, experiencing similar human-induced changes in their environment show parallel changes in their sexually selected traits. The threespine stickleback is an ecologically flexible species that has adapted to a range of freshwater habitats from its marine origin (Schluter and McPhail, 1992; McKinnon and Rundle, 2002; Hohenlohe *et al.*, 2010; Schluter *et al.*, 2010). In the Gulf of Finland in the Baltic Sea, where the species is common, the environment has changed during the last decades owing to human-induced eutrophication. Primary production increased in the 1970s and the 1980s, but has remained relatively constant since the 1990s (Raateoja *et al.*, 2005). The magnitude of the changes differs among areas, because of variation in the input of nutrients and in water exchange. This spatial heterogeneity could have favoured a differentiation of stickleback populations in relation to local conditions. Molecular work reveals weak neutral genetic differentiation of populations in the Baltic Sea ($F_{\mathrm{ST}} = 0.003$), but no neutral genetic differentiation in the Gulf of Finland (Leinonen *et al.*, 2006; Mäkinen *et al.*, 2006). Yet, morphological differentiation is recorded in the Gulf of Finland, which indicates that the populations can adjust phenotypically to local conditions, although the degree to which this is genetically determined is unknown (Leinonen *et al.*, 2006).

We selected parallel pairs of mildly and severely eutrophied habitats in the Gulf of Finland and recorded courtship behaviour and red coloration of males breeding in the habitats. The recordings were done under standardized conditions. We chose two of the areas investigated in Leinonen *et al.* (2006), between which weak morphological divergence was recorded, and within each area we selected two habitats: one with good water quality and another where water quality has deteriorated because of human activities during the last decades. Stickleback move in spring from deeper water to coastal waters to breed, primarily to spatially separated shallow bays. The system thus offers an opportunity to investigate parallel differentiation of sexually selected traits in response to an anthropogenic disturbance.

METHODS AND MATERIALS

Populations

We collected stickleback during the week the breeding season started from two areas in the Gulf of Finland, separated by 220 km: West-Nyland in the western part and Kotka in the eastern part. In each area, we selected a clear water habitat (C) and a eutrophied habitat (E), separated by 5–11 km. The habitats are classified to be in good versus poor ecological state based on long-term observations of several environmental factors (Vuori *et al.*, 2009). Primary production and water clarity have differed between the habitats during the last decades (Table 1). The fish were brought to Tvärminne Zoological Station and housed in large flow-through tanks with clear sea water under natural light conditions, with the sexes separated, for a maximum of 4 weeks.

Courtship behaviour

Males with hints of nuptial coloration were housed in individual 10-L flow-through aquaria containing a nesting dish filled with sand, filamentous algae (*Chladophora glomerata*) for

Table 1. Chlorophyll *a* concentration and Secchi depth (mean ± S.D. across years) during the last decades in the clear water (C) and eutrophied (E) habitats in the two areas, Nyland and Kotka

	Nyland		Kotka	
	C (1979–)	E (1993–)	C (1972–)	E (1972–)
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	3.7 ± 1.91	5.6 ± 2.38	4.7 ± 1.60	8.2 ± 2.95
Secchi depth (m)	4.2 ± 1.47	2.6 ± 0.84	3.0 ± 0.71	2.0 ± 0.45

Note: The measurements were taken once a year during the breeding season (June in Nyland, July/August in Kotka). Year indicates when the measurements were begun; last measurements are from 2010. Data were compiled by the Finnish Environmental Administration (http://wwwp2.ymparisto.fi/scripts/oiva.asp).

nest construction, and an artificial plant for cover (Candolin, 1997). Males were presented with a gravid female from their own population, enclosed in a glass jar, twice a day for 10 min to stimulate nest building. The nest was considered ready when it had a clear nest entrance and the male had performed ‘creeping through’ behaviour (Barber *et al.*, 2001).

As soon as a male had completed nest building, the male and the nesting dish were moved to an experimental aquarium (70 × 30 × 30 cm). The aquarium was divided into a male (55 × 30 × 30 cm) and a female (15 × 30 × 30 cm) section separated by a perforated Plexiglas divider, which allowed visual and olfactory contact. Bunches of artificial filamentous algae (Candolin *et al.*, 2007) were distributed over the bottom, mimicking a moderately vegetated habitat.

After one day of acclimatization, the male was presented first with a dummy female and, after a one-hour break, a live gravid female from his own population. A dummy female was used to standardize female appearance and prevent variation in female behaviour from influencing male behaviour. Each female presentation lasted 20 min. The individuals were video-recorded from above. The following components of courtship were recorded from the videos: latency to courtship, number of zigzag bouts (swims in a series of horizontal dashes towards the female), number of leads (attempts to lead the female to the nest in a direct movement), number of times creeping through or over the nest (the male swims through the nest after the lead to the nest, or, alternatively, swims over the nest with similar movements as when creeping through), number of bites on the female, time spent fanning at the nest entrance (the male fans fresh water into the nest with his pectoral fins), and time spent on nest maintenance (cleaning and gluing the nest). When the male did not court the female, latency to courtship was noted as the total recording time, 20 min. The wet mass (±0.1 g) and the standard length (±1 mm) of the males were measured after the recordings. Condition was estimated as residual body mass, calculated from regression of wet mass on standard length, using both untransformed and log-transformed values. Qualitatively similar results were obtained in the analysis using the two measures, so only results for untransformed values are presented.

Red coloration

The lateral sides of the males were photographed immediately after the female presentations, both after the dummy presentation and after the live female presentation, using standardized methods (see Candolin, 1999; Heuschele *et al.*, 2009). The male was dip-netted and placed in a small glass box containing water and a black sponge that fixed him in place. The glass box was in turn placed inside a black, non-reflective box containing a digital camera and both lateral sides of the male were photographed. Total and relative areas of red coloration were calculated from the digital images using the image processing software ImageJ v.1.44 (http://rsb.info.nih.gov/ij/) and the plug-in ‘Threshold Color’. We used the HSB colour model because the stickleback visual system resembles that of humans (Rowe *et al.*, 2006). Red areas were selected using the following values: hue = 0–21 and 238–255, saturation = 83–255, and brightness = 0–255. The images were converted to 8-bit grey-scale and the selected areas were measured using the ‘Image/Adjust/Threshold’ and ‘Analyse/Measure’ commands. The total area of the fish was measured to calculate the relative area of red. The quality of the red colour of the selected areas was calculated as $R/(R + G + B)$ (Barber *et al.*, 2000) using values from the plug-in ‘Measure RGB’ of ImageJ. The average values of the two lateral sides were used in the analyses.

Analyses

Only males that performed at least three zigzag movements or leads during the dummy or the live female presentation were considered to have reached the courtship stage. Males performed less courtship with the dummy female, and only total number of zigzags and leads, the most common courtship behaviours, were analysed for the dummy presentation. Courtship behaviours with the dummy female were strongly right-skewed and log $(x + 1)$ transformed; red area and courtship behaviour with live females were slightly right-skewed and square-root $(x + 0.5)$ transformed; and male body length was left-skewed and cube transformed. We used linear and generalized linear mixed-models (REML) with area (West-Nyland and Kotka) as a random factor and habitat (clear, C and eutrophied, E) as a fixed factor. The interaction between the random and the fixed factors was inserted as a random factor into the model, but deleted if the effect was non-significant. We used the software R v.2.11.1 (R Development Core Team, 2010) and the 'lmer' function from the lme4 package for generalized linear mixed-models, using the logit link function for binary response variables.

RESULTS

Males from clear and eutrophied habitats did not differ in body size (standard length: $F_{1,135} = 2.89$, $P = 0.091$; wet weight: $F_{1,135} = 1.93$, $P = 0.17$) or condition ($F_{1,135} = 0.33$, $P = 0.56$), or in the likelihood of courting (GLMM, binomial distribution, $N = 138$, $z = 0.37$, $P = 0.71$). Of the males that courted (96 males), those from eutrophied habitats performed more courtship towards the dummy female (Table 2, Fig. 1). Males from the two habitats did not differ in latency to courting the dummy female or in red coloration (Table 2, Fig. 1).

Males performed more courtship with the live female than with the dummy female, and the difference was more pronounced for males from eutrophied habitats (sum of number of zigzag bouts and leads: LMM with male identity as additional random factor, habitat* female identity: $F_{1,94} = 9.24$, $P = 0.003$). When courtship towards the live female is inspected, males from eutrophied habitats performed more courtship, both in terms of the most frequent courtship components – zigzag bouts, leads, and time spent fanning – and for the first principal component of the recorded behaviours, which captures courtship intensity (Tables 2 and 3, Fig. 2). No significant difference was recorded for creeping through or over the nest, which was an infrequent component of courtship, and only a tendency was detected for bites, which represents aggressive behaviour and correlates negatively with courtship behaviour (Tables 2 and 3, Fig. 2). Males from the two habitats did not differ in latency to courting, time spent on nest maintenance or in red coloration (Table 2, Fig. 2).

DISCUSSION

Males from the two areas with paired habitats showed parallel differentiation of courtship behaviour in response to recent human-induced eutrophication. Males from the eutrophied habitat courted the dummy and the live female more intensively than did males from the clear water habitat. Moreover, males from the eutrophied habitat increased their courtship activity more than males from the clear water habitat when presented a live female after the dummy female. This parallel differentiation appears adaptive based on earlier results: males

Table 2. Influence of eutrophication on courtship towards a dummy and a live female (separate linear mixed models with area as a random factor and eutrophication as a fixed factor)

	Eutrophication		Area	
	$F_{1,93}$	P	Wald	P
Dummy female				
Latency	1.22	0.271	0.42	0.674
Courtship	5.66	**0.019**	0.32	0.745
Absolute red area	0.06	0.799	0.31	0.756
Relative red area	< 0.01	0.960	0.46	0.649
Colour quality	0.22	0.639	0.60	0.547
Live female				
Latency	1.51	0.222	0.14	0.885
Zigzags	6.45	**0.013**	0.58	0.559
Leads	5.94	**0.017**	0.63	0.528
Fanning	5.60	**0.020**	0.63	0.531
Creeping	0.62	0.431	0.60	0.547
Bites	3.44	0.067	0.12	0.908
Principal component	6.08	**0.016**	0.63	0.529
Nest maintenance	0.74	0.393	0.59	0.558
Absolute red area*	0.09	0.769		
Relative red area	0.28	0.599	0.31	0.754
Colour quality	0.60	0.439	0.50	0.620

Note: All interactions between the fixed and the random factors were non-significant and deleted from the models.
* No statistics are given for the random factor (area) when the variability between areas was not sufficient to warrant incorporating a random factor in the model.

Table 3. Summary of principal components analysis of courtship towards a live female across the four populations

	Principal component	
	PC1	PC2
Zigzags	0.959	0.079
Leads	0.971	0.031
Time fanning	0.622	−0.153
Creeping	0.851	−0.272
Bites	0.229	0.964
Variance explained	60.5%	20.7%

in clear water have to limit their conspicuous courtship activity because of high visibility to predators (Candolin and Voigt, 1998), while males in turbid water have to increase their courtship activity to be noticed by females and maintain their interest under the reduced visibility (Engström-Öst and Candolin, 2007). Thus, the parallel differentiation of courtship activity suggests that local populations can differentiate adaptively in response to rapid human-induced eutrophication.

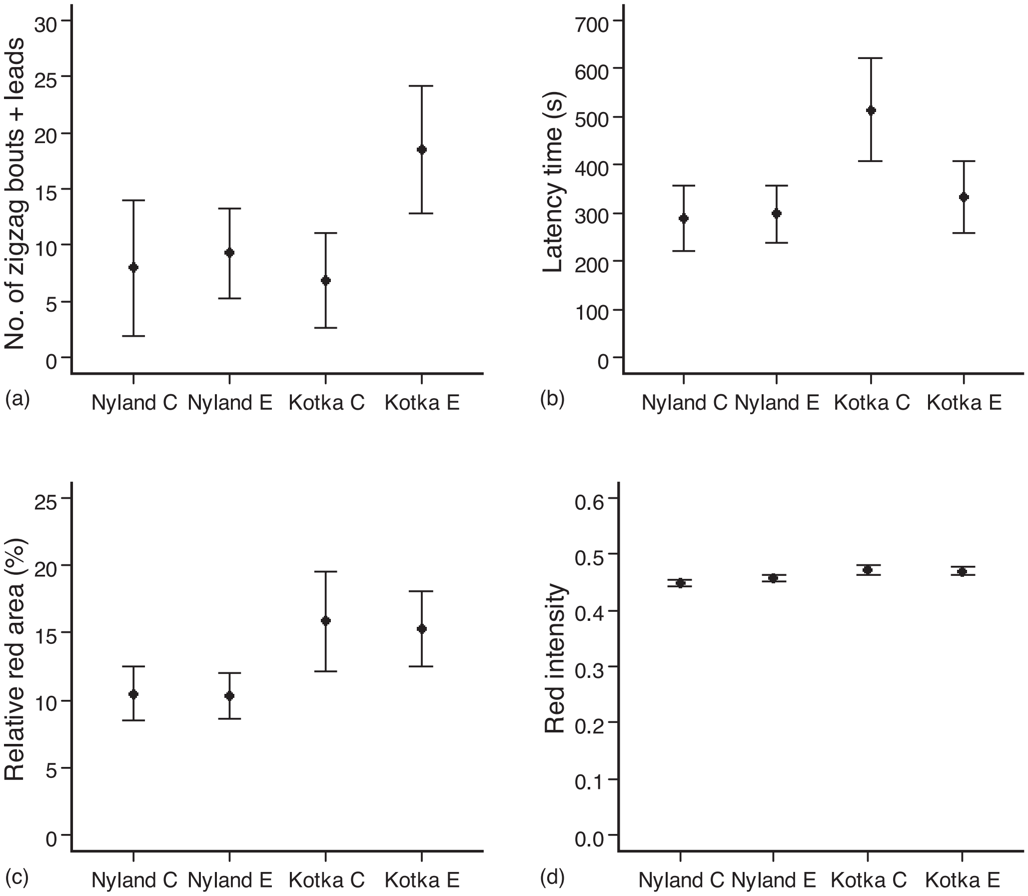
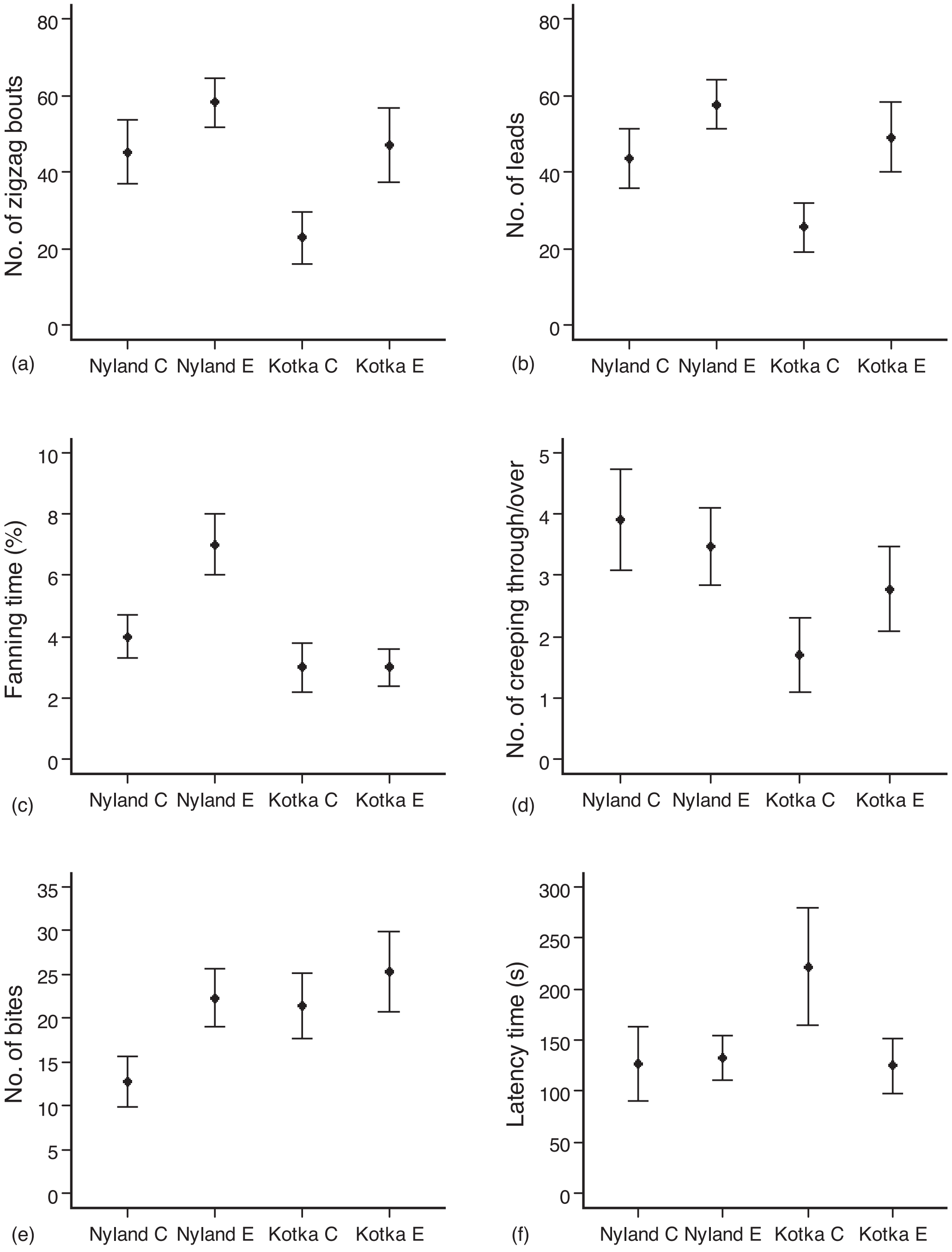


Fig. 1. Mean (± s.e.) trait values of males courting a dummy female for 20 min. (a) Courtship activity, (b) latency time, (c) relative red area, and (d) red colour quality of males from clear water (C) and eutrophied (E) habitats in the two areas, Nyland and Kotka. Values are untransformed.

This parallel differentiation of behaviour could be a plastic response to similar changes in the environment or it could reflect a genetic divergence, or both. There is no barrier to gene flow between the paired populations, and F_{ST} values suggest no neutral genetic differentiation between the two areas (Leinonen *et al.*, 2006). Yet, a divergence in morphology exists between Nyland and Kotka (Leinonen *et al.*, 2006), which suggests that populations in the Gulf of Finland can phenotypically adjust to local conditions. Genetic divergence can occur in less than 20 generations (Stockwell *et al.*, 2003; Hendry *et al.*, 2007; Labonne and Hendry, 2010), and the eutrophied conditions have stayed constant for about 10 generations (the sticklebacks reproduce when 2 years old), which suggests that we could be witnessing the beginning of genetic divergence under gene flow. Adaptive divergence of selected traits can occur even if no differentiation is detected in neutral markers (Labonne and Hendry, 2010; Thibert-Plante and Hendry, 2010). In support of this, ecological factors have repeatedly been found to drive genetic divergence of sexually selected traits in stickleback populations, even for sympatric populations (McPhail and Hay, 1983; Boughman *et al.*, 2005; Olafsdottir *et al.*, 2006; Rayemaekers *et al.*, 2010). For


(a)
No. of zigzag bouts
0
20
40
60
80
Nyland C
Nyland E
Kotka C
Kotka E
(b)
No. of leads
0
20
40
60
80
Nyland C
Nyland E
Kotka C
Kotka E
(c)
Fanning time (%)
0
2
4
6
8
10
Nyland C
Nyland E
Kotka C
Kotka E
(d)
No. of creeping through/over
0
1
2
3
4
5
Nyland C
Nyland E
Kotka C
Kotka E
(e)
No. of bites
0
5
10
15
20
25
30
35
Nyland C
Nyland E
Kotka C
Kotka E
(f)
Latency time (s)
0
50
100
150
200
250
300
Nyland C
Nyland E
Kotka C
Kotka E

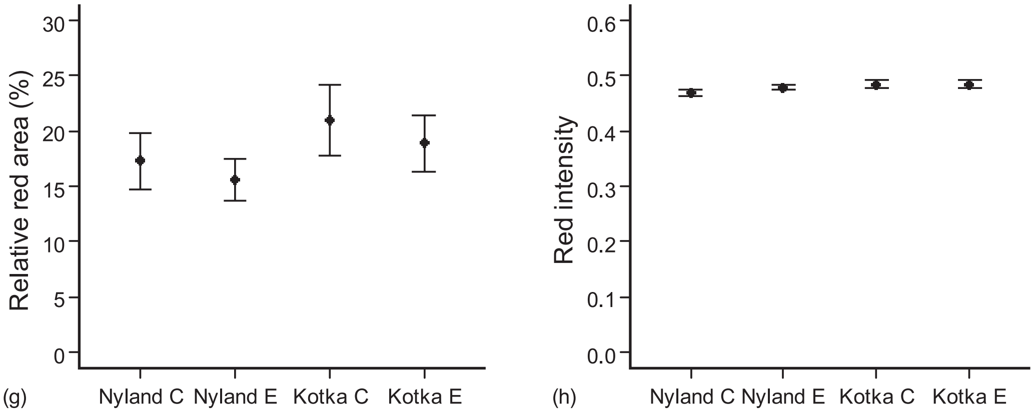


Fig. 2. Mean (±S.E.) trait values of males courting a live female for 20 min. (a) Number of zigzag bouts, (b) number of leads, (c) time spent fanning, (d) number of times creeping over or through the nest, (e) number of bites, (f) latency time, (g) relative red area, and (h) red colour quality of males from clear water (C) and eutrophied (E) habitats in the two areas, Nyland and Kotka. Values are untransformed.

instance, in the Misty watershed on Vancouver Island, parapatric lake and stream stickleback populations have genetically diverged in several courtship components, with lake males courting more aggressively than inlet males, which in turn have a more display-oriented courtship (Delcourt *et al.*, 2008; Raeymaekers *et al.*, 2010). In lakes of northwestern North America, sympatric stickleback populations form a benthic–limnetic continuum, where limnetic males perform conspicuous courtship while benthic males have a less conspicuous courtship (Shaw *et al.*, 2007; Foster *et al.*, 2008). In our parallel populations, the magnitude of the differentiation of the courtship behaviours is within the variation in genetic differentiation reported in these studies, which indicates that genetic divergence could potentially be a cause of the differentiation detected in our populations.

Environmental effects could have reinforced a genetic divergence of courtship, or they could be the sole cause of the differentiation. Environmental effects could arise during the juvenile stage – if individuals return to their natal area to breed after spending the winter in deeper water – or, alternatively, they could arise on arrival at the breeding grounds. In the Gulf of Finland, stickleback arrive at the breeding grounds 2–3 weeks before they start to breed (Candolin and Voigt, 2003). It is plausible that behavioural plasticity is high at the dispersal stage, but reduced when settling into the breeding habitat. This would maximize the benefit of plasticity while reducing its cost (DeWitt *et al.*, 1998). The plasticity could also buffer the population against genetic sources of variation under gene flow (Gibson and Wagner, 2000).

A parallel differentiation of female traits in response to eutrophication could have amplified the differentiation of male courtship behaviour, as the effect of the habitat of origin on courtship was more pronounced with the live female than with the dummy. Males could have recognized females from their own population and adjusted their courtship accordingly, or they could have responded to differences in female traits between populations, such as behaviour or morphology, independent of the origin of the female. Previous research shows that males can recognize females from their own population (Kozak *et al.*, 2009), and that they respond to differences in female behaviour independent of the origin of the female (Delcourt *et al.*, 2008; Raeyemaekers *et al.*, 2010; Räsänen *et al.*, 2012). A differentiation of female traits could be plastically induced or genetically determined, or both, similarly to the differentiation of male traits. Females from the Nyland clear water area do adjust their preferences to environmental conditions (Heuschele *et al.*, 2009), which suggests that plastic differences in female behaviour could have contributed to the differentiation of male courtship. Mate preferences of females could also have diverged genetically in relation to ecological factors (e.g. Boughman, 2001; McKinnon *et al.*, 2004), although the evolution of female preferences is predicted to be slow and reduce rather than increase the rate of a genetic divergence of male sexually selected traits (Labonne and Hendry, 2010). Regardless of the cause of the differentiation, the parallel differentiation of the paired populations in the two areas suggests that threespine stickleback populations differentiate predictably in reproductive behaviour in relation to human-induced environmental change.

Factors other than poor visibility to females could have contributed to the higher courtship activity of males in eutrophied environments. Poor visibility could have reduced conspicuousness to predators, as the main predators in the investigated areas are visual predators: the main predators of adults are perch (*Perca fluviatilis*) and terns (*Sterna hirundo* and *S. paradisaea*) (U. Candolin, personal observation), while the main predators of eggs are conspecifics (Shaw *et al.*, 2007; Foster *et al.*, 2008). Thus, the reduced visibility could have reduced the predation risk cost of courtship. In addition, the higher primary production could have increased the energy intake of males and allowed a larger investment in courtship. Moreover, the difference in courtship activity could be physiologically induced if males from eutrophied and clear water differ in hormonal levels or have experienced different mean temperatures before breeding. It is also conceivable that the difference is a response to the experimental treatment, as males from eutrophied waters experienced a larger improvement in visibility than males from clear water. These explanations still imply an effect of the habitat of origin on courtship activity.

Thus, our results show that anthropogenic disturbances can cause a differentiation of local populations when the disturbance varies spatially. This contrasts with the large number of studies that have found human activities to homogenize populations and cause hybridizations that reduce biodiversity (Seehausen, 2006; Seehausen *et al.*, 2008; Gilman and Behm, 2011; Villeger *et al.*, 2011). In Enos Lake in British Colombia, for example, human-induced environmental change appears to be the cause of hybridization between a species pair of sticklebacks (Taylor *et al.*., 2006). Our results demonstrate that the opposite pattern can occur when human activities increase spatial variation in environmental conditions. If the differentiation has a genetic basis, this could prevent the erosion of genetic variation. Similarly, if the differentiation is a plastic response to environmental condition, this could preserve genetic variation by relaxing genotype–phenotype correlations and shielding genetic variation from selection. Thus, the differentiation of behaviour could maintain high levels of genetic variation, which could be favourable if the environment continues to change and genetic adaptation becomes crucial.

Whether the populations in the Gulf of Finland will evolve into distinct ecomorphs is unknown. The continuous variability in environmental conditions, depending on water flow and nutrient influx, should favour populations that differ to various degrees in their phenotypes. Together with gene flow, this could prevent the genetic differentiation of populations in different areas into distinct morphs. Stickleback populations in the Baltic Sea are currently growing (Ljunggren *et al.*, 2010) and it is possible that the phenotypic adjustment of courtship behaviour to local eutrophication has contributed to their success.

In conclusion, our results show that stickleback populations exposed to similar changes in their environment because of anthropogenic disturbances show parallel differentiation of courtship behaviour. This differentiation appears adaptive, which suggests that the species can adjust predictably to human-induced eutrophication. This could maintain diverse populations, both phenotypically and genetically, and buffer them against further changes to the environment.

ACKNOWLEDGEMENTS

We thank Tvärminne Zoological Station for providing working facilities and Juha Merilä and Bob Wong for comments on the manuscript. This work was funded by the Academy of Finland (to U.C.).

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