Behavioural responses to anthropogenic disturbances

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Academic dissertation

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Abstract

When a habitat undergoes change, the first response of an individual is often behavioural adjustment. This immediate response can determine whether the population will survive or not, as behavioural flexibility gives time for genetic changes to arise later on. Habitat changes that alter reproductive behaviours can have long-lasting effects on populations. If the selective regime has changed under the new conditions, mate choice cues may no longer reliably reflect an individual’s quality. Thus, animals have to be able to adjust their reproductive behaviours to the local conditions.

The aim of my thesis was to discuss if and how animals are able to respond to rapid anthropogenic environmental change, and to study the mechanisms of the responses and the evolutionary consequences. The main focus was on the effects of human-induced eutrophication on the reproductive behaviour of fishes. Eutrophication is the result of increased nutrient input and can cause dense underwater vegetation and algal blooms.

I used fishes from two very different ecosystems as model species, the Baltic Sea threespine stickleback (*Gasterosteus aculeatus*) and the desert goby (*Chlamydogobius eremius*), an endemic species of the Lake Eyre region in Central Australia. I investigated the effects of increased habitat complexity on courtship behaviour and the possibility of local differentiation in courtship and nest building behaviour depending on the level eutrophication in the habitat of origin. Furthermore, I observed the effect of turbidity on stickleback nest building behaviour.

The results show that threespine stickleback males, which were born in areas that have been eutrophied for decades, court females at a higher intensity than males from clear water areas. Similarly, male desert gobies increased their courtship effort in dense vegetation. Intense courtship could be an adjustment to reduced visibility and lowered predation risk in the densely vegetated sites. However, there were no clear differences in nest building between males from clear and eutrophied areas under standardized conditions. This was expected as Baltic Sea sticklebacks prefer to nest under vegetation cover and are fairly rigid in adjusting their nest characteristics. Nest building was affected by increased turbidity: males built smaller nests with a larger nest entrance in turbid water. The large variation in the magnitude of phytoplankton blooms may require a rapid adjustment of the optimal nest structure to the current conditions.

This thesis highlights the complex interactions that are set-off by human-induced changes in habitats and are followed by the immediate behavioural responses. It also encourages more research to tease apart the phenotypic and genetic components of the observed behavioural differences.
1. Introduction

Anthropogenic environmental change and behaviour

Human-induced environmental changes are often wide-spread and rapid. Examples of large-scale anthropogenic effects are urbanization, habitat fragmentation and deforestation. Change is the norm in natural systems but the faster pace of the human-induced alterations can be challenging for many species. Behavioural adjustments are often the initial response of individuals to change. These can have far-reaching effects on survival, distribution of species and reproductive success, and, ultimately, on biodiversity. In the following, I will first discuss behavioural reaction norms and behavioural plasticity in changing environments and the effects of environmental change on sexual selection and reproductive behaviour. I will then proceed to discuss the effects of human-induced eutrophication on fish reproduction and introduce the model species I used in my experiments - the threespine stickleback (Gasterosteus aculeatus) and the desert goby (Chlamydogobius eremius).

There are three timescales at which behavioural responses can occur at the population level: first is the immediate, individual-level response and adjustment of behaviour, second the adjustment of behaviour through innovations and transmission of information within and across generations, and, finally, the evolution of behavioural reaction norms over generations. In my thesis, I will concentrate on the immediate responses, as these will determine whether the population will survive the initial stage of change.

Behavioural responses to environmental change can sometimes increase the probability of survival and/or enhance reproductive success under the new conditions and thus benefit the population. This also gives time for genetic changes to arise (Pigliucci 2001). However, the responses can be maladaptive as well, and take the population further away from the new adaptive peak on an adaptive landscape (Ghalambor et al. 2007; Crispo, 2008), and under a worst-case scenario, lead to extinction (Badyaev 2005). Individuals originating from evolutionary stable environments can be expected to respond maladaptively to change since it is likely that they are facing conditions they have not encountered before during their evolutionary history (Ghalambor et al. 2007). These individuals generally do not possess the behavioural plasticity needed to overcome the effects of sudden changes.

A common behavioural response to a habitat alteration is dispersal — individuals move to a more favourable environment. This, in turn, will alter species composition in the sink and source of the movement, and affect biodiversity and species interactions. In addition, changes in sexually selected traits can have long-term effects on population viability and evolution (Seehausen, Van Alphen & Witte 1997; Maan, Seehausen & Van Alphen 2010).
Mechanisms of behavioural responses

To be able to predict whether a species will survive in environments undergoing sudden changes requires knowledge of the mechanisms of the responses and their dependence on extrinsic and intrinsic factors. The responses of animals to environmental change depend on individual behavioural reaction norms. Reactions norms can be described as the range of phenotypes produced by a particular genotype in different environmental conditions (Schlichting & Pigliucci 1998). The norms are genetically determined and can evolve over generations through innovations and learning, depending on genetic variation and constraints. Thus, the plasticity of the reaction norms depends on the environment they evolved in: fluctuating environments favour plasticity, but in stable environments plasticity is lost as directional selection leads to fixed behaviours and canalized traits (Pigliucci 2001; Sultan & Spencer 2002). If the environment undergoes a drastic, unpredictable change, the reaction norms needed for survival may not exist. Contemporary evolution of the norms is then the only option for persistence (Van Buskirk & Steiner 2009).

Physiological processes, life-histories, specialization, individual differences and, as mentioned earlier, the rate of change, can affect the ability of species to respond adaptively to change. The rat (Rattus norvegicus) and the house sparrow (Passer domesticus) are good examples of successful habitat generalists (Marzluff 2002; McKinney 2002) that have learnt to exploit food sources provided by humans in urban areas and are aggressive competitors. These species fare well in anthropogenic environments and have become invasive at the expense of the native species.

Reproduction in a changing environment

Changes in environmental conditions can affect several aspects of reproduction, including sexual selection, and thus have long-lasting effects on populations. Sexual selection drives the evolution of traits that improve reproductive success in situations with limited access to gametes of the other sex (Andersson 1994). Mate choice and intra-specific competition, the main components of sexual selection, have costs and benefits that are determined by the abiotic and biotic environment. Thus, to maximize reproductive success, reproductive behaviours have to be adapted to the local environment (Candolin & Salesto 2006). If the local conditions are altered, the selection pressures might change as well.

Mate choice decisions depend on signals provided by the opposite sex. These signals can be either phenotypic traits or resources and should reflect mate quality. The honesty of the signals produced depends on the common interests of the signallers and receivers (Maynard Smith & Harper 2004) or on costs involved in signal transmission (Zahavi 1975). The assessment of
signals and of sexually selected traits can be hampered by environmental change, such as decreased visibility or loud noise. Animals may try to adjust their signals to match the new conditions. For example, great tits (Parus major) sing at a higher minimum frequency in noisy areas to prevent their songs from being masked by low-frequency noise (Slabbekoorn & Peet 2003; Slabbekoorn & Den Boer-Visser 2006).

The most studied phenotypic traits used in mate choice have traditionally been physiological and morphological characteristics. However, in nest building animals, the nest can also function as an extended phenotypic trait, and as a mate choice cue (Schaedelin & Taborsky 2009). A well-known example of an extended phenotype is the elaborate bower of a bower bird (Borgia, Pruett-Jones & Pruett-Jones 1985). The important question here is whether these behaviourally-mediated extended phenotypic traits, i.e. nests, can be similarly adjusted to rapidly changing environmental conditions as behaviour per se.

Local environmental change and the resulting novel selection pressures can result in divergent mate preferences between populations and could eventually lead to assortative mating and thus, prezygotic isolation and even speciation (Schluter 2001). Sexual selection is thus a major force in reinforcing reproductive isolation between separated populations.

Eutrophication and fish mating behaviour

Human-induced eutrophication is a problem affecting many aquatic systems worldwide. It is the result of increased nutrient input, mainly nitrogen (N) and phosphorus (P), from, for example, agricultural runoff and wastewater. Increased primary production can deplete oxygen in the water and have severe ecological impacts, such as hypoxic bottom layers (Bonsdorff et al. 1997), but also more subtle effects, such as decreased underwater visibility through increased growth of phytoplankton and vegetation.

Human-induced eutrophication can affect the use of visual mate choice cues for fish species with visually based mating systems, resulting in changes in mate choice and courtship behaviour (Heubel & Schlupp 2006; Candolin, Salesto & Evers 2007; Engström-Öst & Candolin 2007; Heuschele & Candolin 2007; Heuschele et al. 2009; Sundin, Berglund & Rosenqvist 2010), mating system breakdown (Järvenpää & Lindström 2004) or hybridization between closely-related species (Seehausen, Van Alphen & Witte 1997).

Reproductive behaviours are often highly conspicuous and can increase the risk of becoming predated, especially for males (Husak et al. 2006). Turbidity and dense vegetation provide cover from predators, lowering the cost of courtship and mate choice (Candolin 2004) and ameliorating the conflict
between predator avoidance and reproductive behaviour.

The Baltic Sea, where my stickleback study populations are from, is especially vulnerable to eutrophication due to its shallow depth, large catchment area and narrow links to the North Sea. In the Gulf of Finland in the Baltic Sea, primary production increased in the 1970s and 1980s and levelled off in the 1990s (Raateoja et al. 2005). However, water conditions can differ widely among areas, because of variation in the input of nutrients and water exchange, which could favour local adaptation. Accordingly, the stickleback study system offered an opportunity to investigate local adaptation in response to anthropogenic disturbance.

Anthropogenic disturbances can affect aquatic systems in as isolated locations as deserts. Desert rivers and springs, and the endemic fish species that inhabit them (such as the desert goby), provide another unique system for studying the effects of habitat change on fish reproductive behaviour. Due to the scarcity of freshwater habitats in desert environments, they are facing increasing pressure from anthropogenic sources. In particular, livestock can contribute nutrients to the water through their faeces and urine, promoting dense vegetation growth. The animals also trample and graze the margins of springs (Lucas & Jones 2009). Attempts to manage this by fencing have not been successful: in the absence of the trampling effect the springs are rapidly overgrown by reeds (Kodric-Brown et al. 2007).

The model species and systems

The desert goby

Desert gobies (Chlamydogobius eremius) are endemic to the Lake Eyre Basin of Central Australia and are found in a range of habitats from permanent springs to ephemeral rivers and streams. Male desert gobies nest under rock crevices, court females for spawning and have exclusive paternal care. The male guards and fans the eggs until hatching. Courtship consists of jerky body movements accompanied with the raising of the dorsal and anal fins (fin flares) (Wong & Svensson 2009).

The threespine stickleback

The threespine stickleback (Gasterosteus aculeatus) is a common sight on the coastline of Finland. It is ecologically flexible and has adapted to a range of freshwater environments from its marine origin (Hohenlohe et al. 2010; Schluter et al. 2010). In North America, marine sticklebacks have repeatedly colonized freshwater lakes after the last ice age (McKinnon & Rundle 2002) and today, several sympatric stickleback lineages are known, such as the benthics and limnetics (Schluter & McPhail 1992; Boughman 2001; Rundle & Schluter 2004; Boughman, Rundle & Schluter 2005).

In the Baltic Sea, sticklebacks normally take about two years to mature and experience only one breeding season. The breeding season at our study sites in the south coast of Finland starts in early May and lasts until mid-July.
Sticklebacks breed in shallow coastal waters. Males build a nest on the bottom of the sea out of algae and sand, defend their territory and provide paternal care (Wootton 1976). Nest materials are glued together by a glycoprotein called spiggin (Jakobsson et al. 1999). The male develops bright nuptial colouration that includes blue eyes and a red belly and tries to lead the female to the nest with an intense courtship performance (Wootton 1976).

Courtship behaviour (Kitano, Mori & Peichel 2008), nuptial colouration (Boughman 2001) and even nest design and structure (Rushbrook & Barber 2008; Raeymaekers et al. 2010; Rushbrook et al. 2010) are known to differ between stickleback populations depending on environmental conditions. Previous studies have shown that stickleback males court more intensively in turbid (Engström-Öst & Candolin 2007) and in densely vegetated habitats (Candolin, Salesto & Evers 2007). In addition, male sticklebacks prefer vegetated nesting sites under predation risk and the populations have a higher reproductive output in eutrophied areas (Candolin, Engström-Öst & Salesto 2008). However, highly dense vegetation can have negative effects on visibility (Candolin & Salesto 2006) and relax the strength of sexual and natural selection at the reproductive stage (Candolin, Engström-Öst & Salesto 2008; Heuschele et al. 2009; Heuschele & Candolin 2010).

2. Aims of the thesis

The aim of this thesis was to examine the effects of rapid, anthropogenic environmental change on animal behaviour. More specifically, I focused on human-induced eutrophication and reproductive behaviour and used fishes, the threespine stickleback (Gasterosteus aculeatus) and the desert goby (Chlamydogobius eremius), as model species. Additionally, the aim was to study if local environmental change can alter reproductive behaviour and thus promote divergence between populations.

In Chapter I I review existing literature on anthropogenic environmental change and behaviour on a broader scale. I discuss the causes and patterns of behavioural changes and the possible consequences these might have. I also emphasize the importance of understanding the mechanisms of the responses, their fitness effects and evolutionary implications.

In Chapter II I aim to investigate the effect of changed habitat complexity on courtship behaviour in a desert-dwelling fish, the desert goby. The aim is to determine if and how the males are able to respond to changes in their signalling environment.

Chapter III aims to determine if local divergence in courtship behaviour in response to environmental change is possible in different threespine stickleback populations. In Chapter IV I use the same populations as in Chapter III but I move my focus from courtship
behaviour to possible divergence in nest building behaviour among the populations.

The aim of Chapter V is to study the effects of increased water turbidity on nest building behaviour in the threespine stickleback.

3. Material and Methods

Fish maintenance (II)

The experiment in Chapter II was done in laboratory conditions at Monash University, Australia, during November 2009–January 2010. Desert gobies were collected from the Lake Eyre Basin in Central Australia and transported to the laboratory. The fish were housed in separate-sex 300-L aquaria that were kept at a temperature of 24–26°C and on a 12-h light:dark cycle. The tanks were filled with water maintained at a salinity of 5‰ to mimic field conditions and the fish were fed daily with frozen brine shrimps (Artemia sp.) and commercial fish pellets.

Fish maintenance (III–V) and algal culture

Experiments III–V were done at Tvärminne Zoological Station (60°N, 23°E), University of Helsinki, during the summers of 2009 and 2010. Threespine sticklebacks were caught using seine nets and minnow traps at the start of the breeding season in early May and transported to outdoor facilities at the field station. The fish were housed in large holding tanks with a flow-through of seawater pumped from outside the station for a maximum of 4 weeks and fed with frozen chironomid larvae twice a day. Sexes were separated based on hints of nuptial colouration and developing ova.

Males with nuptial colouration were transferred to individual 10-L aquaria. We provided them with a nesting dish filled with sand and an excess amount of filamentous algae (Chladophora glomerata) and an artificial plant for cover (Candolin 1997). Depending on the experiment, the males were also given threads they could use as ornamentation in their nest (Chapters IV, V). Each male was presented with a gravid female from his own population twice a day to encourage nest building. After finishing the nest, the male was either moved to an experimental aquarium (III) or photographed, measured and released (IV, V).

Turbidity for the experiment in Chapter V was created by culturing a fast-growing non-toxic flagellate algae, Isochrysis sp., which is a part of the phytoplankton community of the Baltic Sea. The 60-L cultivation tanks contained sea water with nitrogen and phosphorus supplements.

Study sites (III, IV)

In Chapters III and IV I studied behavioural differentiation in response to local human-induced eutrophication in four different threespine stickleback populations in the Gulf of Finland. The study sites were chosen based on Leinonen et al. (2006) and on long-term
data of several environmental factors, including chlorophyll-a and Secchi depth (Table 1, Vuori, Mitikka & Vuoristo 2009), provided by the Finnish Environmental Administration. We collected the fish from two areas, separated by 220 km: West-Nyland in the western part and Kotka in the eastern part. Within both areas, we selected a clear water habitat (C) and a eutrophied habitat (E), separated by 5–11 km.

The molecular work presented in Leinonen et al. (2006) indicates weak neutral genetic differentiation in the Baltic Sea \( F_{ST} = 0.003 \) ([Leinonen et al. 2006; Mäkinen, Cano & Merilä 2006]) and no neutral genetic differentiation in the Gulf of Finland. Thus, local adaptation seems to be restricted by high levels of gene flow. However, a slight morphological divergence between the areas does exist.

### Table 1. Mean (±SD) chlorophyll-a concentration and Secchi depth in the last decades for the study populations in Chapters III and IV. C = clear water, E = eutrophied.

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<td>E (1993-)</td>
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<tr>
<td></td>
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<td>5.6 ± 2.38</td>
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<tr>
<td>(µg/l)</td>
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<tr>
<td>Secchi depth (m)</td>
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<td>2.6 ± 0.84</td>
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Courtship experiments (II, III)

In Chapter II I investigated the effect of complex habitat structure on male desert goby courtship behaviour. We used experimental aquaria measuring 20 × 40 × 65 cm. Each aquarium was divided into a larger male compartment (20 × 40 × 55 cm) and a smaller female compartment (20 × 40 × 10 cm). The two sections were separated by a perforated Plexiglas divider which allows the transmission of both visual and olfactory cues. We had a complex and an open habitat treatment and the experimental design was paired, so each male was subjected to both treatments, in random order. In the complex habitat treatment 12 bunches of bulrushes were added to the test tank and in the control treatment the tank was left bare.
The males were allowed to acclimatize for 5 days before the start of the trial. A gravid female was put into her section of the tank one day before the trial for acclimatization. We recorded the time it took the male to leave his nest and approach the female (latency time) and started behavioural observations once the male had swum within 5 cm of the female compartment. Specifically, we conducted spot samples every 10 s over a 10 min sampling period (Wong & Svensson 2009) and observed the frequency of courtship behaviours.

In Chapter III, threespine stickleback males from four different populations in the Gulf of Finland were observed courting a dummy and a live female under standardized conditions. The areas we used have differed in the degree of eutrophication for decades. After the males had finished building a nest in their individual nesting tank, 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, as above, by a perforated Plexiglas divider. The aim was to mimic a moderately vegetated habitat and bunches of artificial filamentous algae were placed on the bottom of the tank (Candolin, Salesto & Evers 2007).

The male was left to acclimatize for one day and then presented first with a dummy female and, after a 1-h break, with a live female from his own population. Each presentation lasted 20 min. The trials were video-recorded from above. The following behaviours were observed from the videos: latency to courtship, time spent on nest activities, number of zigzag bouts, leads, bites and nest creepovers and creepthroughs. Video material was analysed using JWatcher – video analysis software (http://www.jwatcher.ucla.edu/).

Nest building experiments (IV, V)

In Chapters IV and V the aim was to investigate possible local divergence in threespine stickleback nest building behaviour in response to eutrophication (IV) and the effects of turbidity on nest design and structure (V). I used the same populations in Chapter IV as in Chapter III. As mentioned earlier, males with hints of breeding colouration were housed in individual 10-L nesting tanks with a flow-through of sea water (IV) or standing sea water (V). In Chapter V, the water in the nesting tanks was either clear or turbid (algae-rich), and all males experienced both treatments in random order. In addition to a nesting dish with sand and filamentous algae, the males were provided with 70-mm long red, yellow, blue, and green threads, ten of each, placed as far from the nesting dish as possible (approx. 30 cm distance) (IV) or twenty 70-mm long red threads (V) to use as decorations in nest building. Sticklebacks will readily accept threads as building material and males are known to use decorations in their nests (Östlund-Nilsson & Holmlund 2003).
Gravid females (from the male’s own population, Chapter IV) were presented to the males to stimulate nest building. The nest was considered ready when a nest entrance was visible and the male had performed creeping through or creeping over behaviour (Barber, Nairn & Huntingford 2001).

After finishing the nest, the male was removed from the tank, measured, and photographed. The nest was photographed directly from above and placed on a dry, flat surface for drying. Once the nest had dried, it was photographed again against a white background with a ruler for calibration, weighed and the number of threads incorporated in it was counted. We measured the total and bulk area of the nests from the photographs using image analysis software (ImageJ version 1.44 [http://rsb.info.nih.gov/ij/]) and calculated a neatness index ($I_n$, the proportion of visible thread ends and loose ends of algae that were not glued to the nest), according to Barber et al. (2001). We also used the photographs for observing whether the threads were used as decorations at the nest entrance or as building material (incorporated into the nest). In Chapter V, after the first treatment, the experimental aquarium was cleaned and filled again with either clear or turbid water, depending on the treatment that was applied first. The male was returned to the tank the next day and the whole process was repeated. Males were photographed after both treatments, but measured only after the second one.

Fish traits (II–V)

Wet mass ($\pm 0.01$ g) and standard length ($\pm 1$ mm) of all males (II–V) and females (II, V) used in the experiments were measured after the trials. In Chapter II, desert goby males and females were photographed from above on top of a grid in a small plastic box filled with water and the length of the fish was measured from the photographs (ImageJ version 1.44). The extent of the red nuptial colouration of threespine stickleback males was estimated by photographing both lateral sides of the male and calculating the total and relative red area from the photos using ImageJ and following standardized methods (Candolin 1999; Heuschele et al. 2009). We used the average of the two lateral sides in the analysis.
4. Main results and discussion

Effects of anthropogenic disturbance on courtship behaviour (II, III)

The results in Chapter II show that there was a tendency for larger desert goby males to have a shorter latency time. In addition, irrespective of size, desert goby males courted more in a complex habitat. Males also spent more time in the nest in the open habitat treatment (Figure 1A and 1B). Males could be compensating for the reduced visibility by courting more. On the other hand, the increased cover allows more conspicuous behaviours.

Figure 1. Mean (± S.E) number of times male gobies were observed (A) engaging in courtship activity and (B) inside the nest in open versus complex habitats.

Courtship experiments with threespine sticklebacks showed similar results (III): males from eutrophied habitats courted more actively than males from clear water habitats (Figure 2) – as did desert goby males in dense vegetation. Intensive courtship may be required in eutrophied areas to gain the same amount of interest from the females as in clear water (Engström-Öst & Candolin 2007). Thus, in the light of earlier research, the results seem adaptive (Kraak, Bakker & Mundwiler 1999).

In addition, the perceived predation risk, from both predators (Candolin & Voigt 1998) and cannibalistic conspecifics (Shaw, Scotti & Foster 2007), is lower in habitats with poor visibility and dense vegetation cover. High turbidity reduces the reactive distance of visual predators to their prey, which in turn reduces their foraging efficiency - for example in species such as the brown trout (Salmo trutta) (Stuart-Smith, Richardson & White 2004) and Eurasian perch (Perca fluviatilis) (Radke & Gaupisch 2005; Ljunggren & Sandström 2007). Males
are often more susceptible to predation than females due to their bright nuptial colouration, conspicuous behaviour and larger size (Stuart-Fox et al. 2003; Husak et al. 2006). Dense vegetation enables the males to increase their signalling effort at a lower ultimate cost.

Primary production is higher at eutrophied sites. This could allow more investment into courtship as energy intake is probably also higher and as the lower predation rate in low visibility conditions allows not only intense courtship, but also increased feeding (Gregory & Northcote 1993). However, sticklebacks are visual predators themselves and if predation success is dependent on visual cues, high turbidity and/or dense vegetation can reduce their food intake rate (Engström-Öst et al. 2007), as well as that of their predators, making the situation more complex. In addition, some physiological aspects could influence the responses, such as hormonal levels or temperature at the habitat of origin, although a lack of differentiation in body size and condition among the males does not support this.

Figure 2. Courtship activity towards a live female in Nyland and Kotka populations. C = clear, E = eutrophied.

The study sites we used in Chapters III and IV have stayed at the same level of eutrophication for at least 10 generations. Although gene flow is strong, divergence in courtship behaviour and, eventually, genetic divergence, could happen as genetic differentiation can be rapid and take place in less than 20 generations (Stockwell, Hendry & Kinnison 2003; Labonne & Hendry 2010). Furthermore,
sticklebacks normally return to their natal area to breed. Thus, if plasticity is highest during the dispersal back to the native breeding areas and decreased when they settle into a habitat (DeWitt, Sih & Wilson 1998), it could buffer the populations against genetic sources of variation.

Effects of anthropogenic disturbance on nest building (IV, V)

Chapters IV and V focus on another component of sexual selection in many species: the nest. The purpose of a nest is to function as protection for the offspring against harsh environmental conditions and predation (Hansell 2000). These conditions can vary from one habitat to another and the changes can be human-induced and rapid. However, the design and structure of a nest affects not only offspring survival (Wootton 1976), but also mate attraction (Barber, Nairn & Huntingford 2001). Thus, the nest can be viewed as an extended phenotype that is shaped by both natural and sexual selection. As seen in Chapters II and III, both desert gobies and threespine sticklebacks are able to respond to changes in their breeding environment by adjusting their courtship behaviour. The aim here was to determine whether extended phenotypes, i.e. the nest, are adjusted to changing conditions.

Threespine stickleback populations have been observed to vary in their nest building depending on environmental conditions (Rushbrook & Barber 2008; Rushbrook et al. 2010). River populations are known to adjust their nest characteristics according to the flow regime (Rushbrook & Barber 2008), while Baltic Sea populations seem fairly rigid in their nest building (Candolin & Salesto 2006). Stickleback males prefer to nest in vegetated areas under predation risk (Kraak, Bakker & Hocevar 2000). Males are also known to have artistic tendencies: they decorate their nests when ornamentations are available and these decorated nests are preferred by the females (Östlund-Nilsson & Holmlund 2003). However, in our study, approximately half of the males, independent of the habitat of origin (Chapter IV: 44%; Chapter V: 57% in turbid water, 43% in clear), used the threads we provided them with as building material (incorporated into the nest, not in clear view), not as decorations at the nest entrance.
Table 2. Characteristics (mean ± SD) of nests built by males from the four populations and test statistics (Chapter IV). Linear mixed models, with habitat (C, E) as a fixed factor and area (Nyland and Kotka) as a random factor, were used in the analysis for all but the last variable. Generalized linear mixed model with a Poisson distribution was used for the total number of threads. C = clear, E = eutrophied.

<table>
<thead>
<tr>
<th></th>
<th>Nyland C</th>
<th>Nyland E</th>
<th>Kotka C</th>
<th>Kotka E</th>
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<tr>
<td>N</td>
<td>28</td>
<td>36</td>
<td>33</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nest weight ± 0.001 g</td>
<td>4.11 ± 2.11</td>
<td>6.57 ± 3.87</td>
<td>5.78 ± 2.79</td>
<td>4.81 ± 3.22</td>
<td>-0.96</td>
<td>0.34</td>
</tr>
<tr>
<td>Total area (mm²)</td>
<td>2037 ± 589</td>
<td>1749 ± 719</td>
<td>1918 ± 574</td>
<td>2000 ± 494</td>
<td>0.94</td>
<td>0.35</td>
</tr>
<tr>
<td>Bulk area (mm²)</td>
<td>1645 ± 565</td>
<td>1413 ± 608</td>
<td>1635 ± 559</td>
<td>1732 ± 496</td>
<td>0.68</td>
<td>0.50</td>
</tr>
<tr>
<td>Neatness index, $I_n$ ($A_{bulk}/A_{tot}$)</td>
<td>0.79 ± 0.10</td>
<td>0.82 ± 0.13</td>
<td>0.85 ± 0.11</td>
<td>0.87 ± 0.09</td>
<td>-1.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Total number of threads</td>
<td>31.82 ± 12.96</td>
<td>28.14 ± 14.78</td>
<td>29.52 ± 13.60</td>
<td>30.60 ± 12.40</td>
<td>-1.08</td>
<td>0.28</td>
</tr>
</tbody>
</table>

In addition, there were no significant differences in nest size (weight and area), neatness or use of ornaments observed between males from clear and eutrophied habitats (Table 2) and no preference for a specific colour as an ornament (IV). Similarly, in Chapter V, there were no differences in the use of decorations between the two treatments. Red is the nuptial colouration of male sticklebacks, and earlier studies have shown that males prefer the colour red as decorative material (Östlund-Nilsson & Holmlund 2003). However, the importance of decorations overall, and the colour red in particular, can be reduced in turbid water and in densely vegetated habitats where visibility is low (Boughman 2001). The high variation in the use of decorations in our study populations indicates weak selection for decorative behaviour in the Baltic Sea. This can be explained by a combination of low visibility of the nests due to the high amount of natural underwater vegetation, and male preference for nesting under vegetation cover (Kraak, Bakker & Hocevar 2000).

Conspicuous nests are favoured by females in some populations (Bell & Foster 1994; Östlund-Nilsson & Holmlund 2003), while in others concealed nests are preferred (Candolin & Voigt 1998; Candolin 1998). Males with concealed nests have also been...
observed to fan and court at a higher frequency than males with unconcealed nests. This could indicate a lower predation risk at concealed nesting sites allowing more intense courtship (Candolin & Voigt 1998). In the Baltic Sea, it seems that it is not the quality or outlook of the nest that is important in attracting a female, but courtship activity (Chapter III).

In chapter V males from only one population were allowed to build nests in both clear and turbid water. The aim was to test the effect of increased underwater turbidity (algal growth) on stickleback nest building behaviour. Males built a bigger nest entrance in the turbid water treatment than in clear water, but the nest itself was smaller, both in area and weight (Figure 3). Nest entrance size could be affected by oxygen conditions in eutrophied areas, especially during the night when respiration can exceed photosynthesis and, as a result, the oxygen levels drop (Engström-Öst & Isaksson 2006). Large amounts of micro algae in the water could also smother the developing eggs. Thus, in eutrophied habitats males would need to fan their eggs more. Sand goby (Pomatoschistus minutus) males in low oxygen conditions have been observed to build nests with larger nest entrances, presumably to make fanning of the eggs easier (Lissåker, Kvarnemo & Svensson 2003). However, in the presence of a predator, sand goby males build a smaller nest entrance, irrespective of oxygen conditions, indicating a trade-off between ventilation and nest defence (Lissåker & Kvarnemo 2006). But, as predation risk is potentially decreased in turbid water, males can build a larger nest opening with a smaller risk of their eggs being predated or eaten by conspecifics than in clear water (Candolin, Engström-Öst & Salesto 2008; Wong, Järvenpää & Lindström 2009).

Males did not adjust the usage or amount of decorations to water turbidity. Thus, adjusting nest characteristics to turbidity conditions (current algal blooms) in the Baltic Sea may be important for offspring survival, but not for mate attraction, as it may not pay off to build a neat and decorated nest if it cannot easily be seen - instead, intensive courtship seems to be required to successfully obtain female interest (Candolin, Salesto & Evers 2007; Chapters II and III). It could be that the importance of the nest as a mate choice cue is reduced in eutrophied areas while the importance of courtship behaviour is intensified.

Males were also faster in building their nest in the clear water treatment than in turbid water in Chapter V, in contrast to Chapter IV, where there was no difference between the populations. This could be explained by the experimental set-up: in Chapter IV, the aim was to test for population differentiation and all males were housed in standardized conditions, i.e. in clear water. For males from eutrophied sites, this could have been the preferred condition, as highly eutrophied areas are not favoured (Candolin & Salesto 2006). This might have sped up the building phase. In the
turbidity experiment (V), all males were from the same population and kept in both clear and turbid water, in random order. The turbid water treatment could then have presented an unfavourable habitat for the males and probably made them more reluctant to start nesting.

As mentioned earlier, stickleback males in the Baltic Sea prefer to nest under vegetation cover and/or concealed between or under stones. Thus, the optimal nest structure does not seem to depend on the density of macro algae or average conditions in the habitat of origin as the visibility of the nest is low and can be assumed to have little effect on female choice and offspring survival (Chapter IV). In addition, if visibility is poor, females could become less choosy towards male quality or the nest (Järvenpää & Lindström 2004). Females can only inspect the nest once at the nest entrance. The final spawning decision may then depend on the presence of eggs in the nest (Forsgren, Karlsson & Kvarnemo 1996) and male characteristics (Candolin, unpublished data). In both chapters IV and V, only about half of the males used the coloured threads as decorations, which further supports the statement. This also agrees with earlier studies (Candolin & Salesto 2006), indicating that Baltic Sea sticklebacks are not very flexible in adjusting their nest characteristics to dense vegetation.

However, although it could be that the density of macro algae cover or average conditions at the habitat of origin have no effect on nest building behaviour, turbidity, i.e. high levels of phytoplankton, does (Chapter V). In the Candolin & Salesto (2006) study, artificial plants were used to mimic eutrophied conditions, in contrast to our study in Chapter V, where we used live algae, which could have an effect on the oxygen conditions and/or smother the developing eggs (as detailed earlier). In addition, the magnitude of algal blooms can vary rapidly from day to day and thus, the ability to reconstruct the nest to match the current conditions could be vital for the survival of the eggs. Earlier studies have shown that nest entrance size in, for example, sand gobies can be highly variable (Svensson & Kvarnemo 2007).

Interestingly, a similar lack of differentiation visible in Chapter IV was not observed in the courtship experiment (III). Regardless of the fact that all males were kept in standardized conditions in the experimental aquaria in the latter experiment, males from eutrophied areas courted more intensively. This reflects how reduced visibility hampers the evaluation of male traits but does not influence the benefit of nest characteristics (as detailed above).
Figure 3. Mean (± S.E) nest entrance size (A), nest weight (B), nest total area (C) and bulk area (D) in the clear and turbid water treatment (Chapter V).
5. Conclusions

Both threespine sticklebacks and desert gobies inhabit areas that have undergone severe changes during their evolutionary history and continue to do so today. Desert springs can form and dry out within decades to centuries (Kodric-Brown et al. 2007) and sticklebacks have migrated from purely marine habitats to freshwater after the last ice age and diverged into several different freshwater forms (Schluter & Mcphail 1992; Bell & Foster 1994; McKinnon & Rundle 2002; Hohenlohe et al. 2010; Schluter et al. 2010). Today, the breeding areas of these fish are faced with increasing disturbance from anthropogenic sources, mainly eutrophication.

Species inhabiting fluctuating environments benefit from high plasticity, even if it could be costly to maintain (van Buskirk & Steiner 2009). Plasticity in behaviour gives time for genetic changes to arise (Pigliucci 2001; Ghalambor et al. 2007; Kinnison & Hairston 2007), especially in situations where the change is large-scale and rapid, like many human-induced environmental changes often are. Hence, behavioural responses are a good starting point for studies that try to determine the effects of sudden changes, their mechanisms and the evolutionary consequences.

Sticklebacks provide plenty of evidence of assortative mating in sympatric populations and of rapid genetic divergence in response to varying environmental conditions (Rundle et al. 2000; Boughman 2001; Boughman, Rundle & Schluter 2005). Stickleback populations in the Baltic Sea are thriving (Ljunggren et al. 2010). One possible reason for this could be the species’ ability to make small adjustments in courtship behaviour according to local environmental conditions. Desert gobies are much less studied but highlight the far-reaching effects of anthropogenic changes and the flexibility of animals living in unstable environments.

Both species seem to be able to behaviourally meet the demands of the changing habitats by increasing their courtship activity (II, III). Thus, at the immediate level, the fish are able to respond to increased habitat complexity, and, in agreement with earlier research, the response seems adaptive (Engström-Öst & Candolin 2007). Extended phenotypes were also affected; males adjusted the size of their nest and of the nest entrance according to turbidity, i.e. the amount of algae in the water (IV), but similar clear differences in nest building between populations originating from clear and eutrophied habitats were not observed under standardized conditions (IV). As the males in our study populations build their nests concealed under vegetation, it is possible that adjustment of nest size, neatness or ornaments would have no effect on mate attraction or offspring survival and thus, males do not match these characteristics to macro algae density or average conditions in their habitat of origin in the Baltic Sea. However, males do adjust the size of the
nest and nest entrance to the magnitude of algal blooms (turbidity) and thus to the prevailing predation risk and oxygen conditions, as this is vital for the survival of the eggs and could be considered adaptive. In eutrophied areas, courtship behaviour may be important in attracting the female to the nest and, at the nest, the presence of eggs, not the outlook of the nest, may determine the female’s final spawning decision.

The results seem to highlight the importance of intense courtship in eutrophied areas in the Baltic Sea while the importance of the nest as a mate choice cue is reduced; the nest is small, loose, has less red ornaments, and the males are reluctant to start nesting when visibility is low (V). Eutrophication could be forcing sticklebacks to nest in unfavoured sites, but at the same time, intense courtship compensates for the poor visibility.

The next step in studying this system would be a common-garden experiment to define the possible genetic basis of the behavioural differences. This would enable us to distinguish between genetic differentiation among the populations, phenotypic plasticity, or some combination of the two. The effect of female behaviour on male behaviour is also an interesting and important question still waiting for an answer.
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7. Literature cited


Kraak, S. B. M., Bakker, T. C. M. & Hocevar, S. 2000. Stickleback males, especially large and red ones, are more likely to nest concealed in macrophytes. Behaviour 137:907-919.

Kraak, S. B. M., Bakker, T. C. M. & Mundwiler, B. 1999. Sexual selection in sticklebacks in the field: correlates of reproductive,


