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1. Introduction

1.1 Sexual selection and mate choice

Males often have secondary sexual ornaments, which are costly for their bearers. Examples of such ornaments are the peacock’s colourful tail, the exaggerated eyestalks of flies in the family Diopsidae, and the giant antlers of the Pleistocene giant elk (Megaloceros giganteus). Darwin suggested that such traits remain in populations for two reasons. Firstly, males would compete with each other to gain access to females, and weaponry (such as antlers) would help them win the fights. Secondly, he suggested that certain traits make males more attractive towards females (Ridley 2004). For these two reasons, trait bearing individuals are more successful at reproducing than others, leading to a selection process that Darwin called sexual selection. The first mechanism, male-male competition, was easily accepted by Darwin’s contemporaries, but the idea of females acting as strategic decision makers raised opposition among researchers for almost a century. If females were actively choosing their mates, this would involve large costs in terms of energy and time spent. It was generally thought at the time that natural selection always acts for the good of the species (Mayr 1972). Based on that assumption it was unlikely that costly traits, such as secondary sexual traits, could be selected and maintained in populations (Andreas 2002). Nowadays the situation is different. Several theories on mate choice evolution have been presented during the past decades, and the field [of sexual selection and mate choice] is currently one of the most active in evolutionary biology research (Andreas 2002).

Mate choice is a common phenomenon and occurs especially in polygynous species where males mate with several females. Females usually spend more energy on gamete production than males and are often the choosy sex (Kokko and Monaghan 2001, Tregenza and Wedell 2000), but not always (Rowell and Servedio 2009, Andreas 2002, Bonduriansky 2001, Reading and Backwell 2007). Female mate choice can have direct benefits (material benefits to the females themselves) or indirect benefits (genetic benefits for the offspring) (Andersson and Simmons 2006, Kokko et al. 2006, Penn 2002, Cothran 2008). Females can profit directly for instance from parental care, food,
increased likelihood of successful fertilization, shelter or protection from predators offered by the male (Seki et al. 2007, Bisazza and Marconato 1988, Kemp et al. 2007). Sometimes there are no material benefits to the female itself, but it may benefit from mating selectively with males that possesses inheritable genes that improve offspring fitness in some way (Neff and Pitcher 2005, Andreas 2002, Møller and Alatalo 1999, Gavrilets et al. 2001). Direct and indirect fitness benefits are not mutually exclusive, but instead, they are both likely to occur (Møller and Jennions 2001).

It is easier to prove that mate choice exists than to show how preferences for mates evolve. Several processes may co-occur, which complicates the situation (Andersson and Simmons 2006). Some of the processes that have gained most attention in literature are Fisher’s runaway process (1930), good genes-model (Zahavi 1975), and genetic compatibility process (Andersson and Simmons 2006, Andreas 2002). Here I briefly review these processes.

Fisher suggested that a self-reinforcing coevolution process may lead to female preferences toward male traits. If female preference and the male trait become genetically coupled, a female choosing a male with a large trait will pass the trait as well as the preference for it to its daughters and sons. Crucial to Fisher’s theory is that there is genetic correlation between female preference and the preferred male ornament (Andreas 2002, Eshel et al. 2002). Even though empirical testing of this Fisherian sexy sons-mechanism is difficult, evidence for it comes from genetic models (Kirkpatrick 1982, Lande 1981) and phenotypic studies (Head et al. 2005, Jones et al. 1998).

The good genes-model, also named as the indicator- or handicap process, is based on the idea that the ornament of the male reflects its good genetic quality (Møller and Alatalo 1999, Heywood 1989, Bell 1978, Zahavi 1975). Genetic quality can be understood in many ways (Hunt et al. 2004), but a high-quality mate is often the one with traits that increase the viability of the offspring for instance in terms of parasite resistance, predator avoidance and growth. The ornament is a reliable signal of quality, if only males with high genetic viability are able to produce and maintain it (Barber et al. 2001). The model assumes that males with the most pronounced secondary sexual
traits have genes that are good for all females, and thus leads to directional selection (Colegrave et al. 2002). If the offspring inherits the good genes, it may have increased viability or other beneficial traits (Møller and Alatalo 1999, Reynolds and Gross 1992). Lek paradox is a problem related to the indicator mechanism that still remains unsolved. If a large trait reflects its bearer’s high quality and females always choose the best individual, a situation follows in which variation in the trait becomes nonexistent. Why then, should females continue to choose if all males are the same quality (Kotiaho et al. 2008, Borgia 1979).

In contrast to the good-genes model, the genetic compatibility process assumes that the best mate to choose is the one whose alleles best complement the chooser (Kempenaers 2007, Colegrave et al. 2002, Brown 1997). Then, what may be a good choice for one female is not necessarily so for another (Brown 1999, Zeh and Zeh 1996). To assess compatibility, females must have some mechanism to distinguish between more and less compatible genotypes. There is some evidence that such assessment can be done after mating (Clark et al. 1999, Wilson et al. 1997). When there is a closer physical association between the gametes within the female reproductive tract, it is easier for the female to distinguish between most compatible sperm cells. Choice based on genetic compatibility could then possibly explain the high occurrence of polyandry, the situation in which one female mates with several males (Tregenza and Wedell 2000). Polyandrous mating in turn may increase the viability or the number of offspring produced (Engqvist 2006, Zeh and Zeh 1996). By choosing the most compatible male, females will optimize offspring heterozygosity and avoid the expression of recessive deleterious alleles and inbreeding depression (Edly-Wright et al. 2007, Penn 2002). The loci of the multihistocompatibility complex (MHC) are thought to be of crucial importance in recognising genetic diversity (Brown 1997). MHC is a very polymorphic locus found in all vertebrates, and it has a key function in immune defense (see below). There seems to be a trend in preference for MHC-dissimilar mates (Jordan and Bruford 1998). The use of MHC cues has been demonstrated mainly in mice, humans and fish (Milinski 2003, Roberts and Gosling 2003).
The indicator mechanism and the genetic compatibility mechanism are somewhat contradictory. If females choose mates based on good genes, mating is random with respect to genetic dissimilarity. The theory of the genetic compatibility mechanism is convincing and there is increasing empirical evidence for it (Mays and Hill 2004), but support for the good genes-mechanism is also prevalent (Tomkins et al. 2004, Zahavi 1975). Even though it may seem paradoxical, it has been shown that both processes can operate within the same species (Colegrave et al. 2002).

Additional processes, to the three mentioned above, have also been suggested. Two of these are direct phenotypic effects, and sensory bias. The phenotypic effects-model suggests that females prefer males that provide them with other than genetic benefits. These benefits include parental care, food, protection from predators, or a high-quality territory (Andersson and Simmons 2006). According to the sensory bias theory, natural selection initially drives female preference for certain male traits for other reasons than mate choice (Andreas 2002). The idea is that a signal receiver may pay more attention to certain signals (such as brighter colour or louder sound), if they are easier to detect. The context of the preference may initially be other than mate choice, e.g. foraging or predator avoidance (Endler and Basolo 1998, Ryan 1998).

There are still ongoing debates among researchers as to which processes are involved in mate choice and to what extent. Recently it has been suggested by Kokko et al. (2006) that Fisher- and good genes-processes are not distinct but a term Fisher-Zahavi process should be introduced.

1.2 Cues and signals in mate choice

Certain phenotypic traits or resources that function in mate choice may evolve, because they increase individual fitness in terms of improved survival. However, they may simultaneously have a role in communication, for example in the process of mate assessment. If this is the case, then in addition to increasing survival, the traits increase the attractiveness of their bearers and improve the individual’s chances of being selected
in mate choice. These kinds of traits, which are evaluated by the choosing sex in mate choice and affect the decision to mate, are called cues. Signals, on the other hand, are cues that have evolved a function in communication. Cues can provide information of the mate quality to the receiver (informative cues), or they may be unreliable indicators of mate quality (uninformative cues). If cues are informative, they potentially facilitate the choice by decreasing its costs which has a positive influence on receiver fitness. When cues are uninformative, they may either increase or decrease receiver fitness, or have no effect on it (Candolin 2003).

Examples of visual cues are colourful ornaments, courtship behaviours and body size, but cues may also be tactile, acoustic, or olfactory (Candolin 2003, Milinski 2003). The use of visual cues has been reported in a wide range of organisms including vertebrates (Reichard et al. 2005, Roberts and Gosling 2003, Reynolds and Gross 1992, Sargent et al. 1998, Bakker and Mundwiler 1994, McDonald 1989) and invertebrates (Costanzo and Monteiro 2007, Backwell and Passmore 1996).

Olfactory cues in turn have been found to be important in mate assessment in several organisms. The multihistocompatibility complex (MHC) is thought essential in transmitting olfactory cues between individuals (Aeschlimann et al. 2003, Brown and Eklund 1994). This gene complex, found in most vertebrates, encodes for proteins that function in the immune defence. MHC-molecules activate T-cells by presenting them peptides from various infecting agents. The allelic combination of the MHC determines which diseases the individual has the resistance for. The evolution of the MHC is rapid, and part of the continuous combat between hosts and their parasites. The hosts are trying to adapt to current conditions by obtaining the combination of alleles that cover resistance for most prevailing diseases, while the prevalent parasites are trying to overcome the hosts’ immune system. MHC can be divided to class I and class II molecules. In teleost fishes the classes are not linked and can be studied separately. It has been found that class I genes encode resistance for intracellular pathogens such as viruses and class II molecules fight extracellular diseases, such as macroparasites (Jäger et al. 2007, Häberli and Aeschlimann 2004, Aeschlimann et al. 2003).
In mate choice, signals may act as multiple messages, and inform the receiver of the different qualities of the potential mate (Møller and Pomiankowski 1993). Multiple messages may help in the evaluation of the overall mate quality. Alternatively, the receiver’s genotype may influence which signals it pays attention to resulting in different individuals assessing different traits (Wedekind 1992). Mate choice typically involves costs in terms of the energy and time spent, and the use of multiple cues may decrease these costs (Candolin 2003, Milinski and Bakker 1992).

1.3 Environmental change altering sexual selection

Environments are changing at an increasingly rapid pace due to human activity, and the ecological effects of the changes are becoming increasingly obvious (Miller and Brooks 2005, Jia et al. 2000, Shykoff and Møller 1999). One alarming example of ecological change is the environment of the Baltic Sea, located between 54° – 66° N and 10° 30’ – 31° E. More than 85 million people inhabit its drainage area and over 100 environmental hotspots within the sea have been identified (Rönnberg and Bonsdorff 2004). As one of the largest brackish water bodies on earth, the Baltic Sea forms a unique ecosystem with distinct fauna and flora. Its deteriorating condition has received much attention in the past decades (e.g. Carpenter 2005, Lissåker et al. 2003, Bonsdorff et al. 2002). One of the main problems concerning the Baltic Sea today is eutrophication, the increased growth of filamentous alga and phytoplankton caused by the increased output of phosphorus and nitrogen (Rönnberg and Bonsdorff 2004). Since the beginning of the 20th century the total nitrogen load increased four fold and phosphorus load eight fold (Larsson et al. 1985). The amount of nutrients that end up in the Baltic Sea from land has decreased since, but the problem has not yet been relieved in the large scale, only locally some improvements have been reported (recent online reports of the Finnish Environment Institute and Finnish Institute of Marine Research).

The problems caused by eutrophication are far-reaching. When excess phosphorus and nitrogen end up in the Baltic Sea, they increase the growth of the rapidly growing filamentous algae and phytoplankton, at the expense of slow-growing macroalgae. This
increases water turbidity, and causes other problems such as anoxic events and changes in pH (Bonsdorff et al. 2002, 1997a, 1997b). Oxygen becomes scarce, because when the organic material sinks to the bottom, anaerobic bacteria become more active, and may use up all the oxygen. The eutrophication cycle is difficult to stop, because even if the nutrient output is reduced, as has happened in the Baltic Sea case, the nutrients stored in the bottom can be reused by plants and the cycle restarts.

In some cases the eutrophication-related changed conditions have forced community structures to reform (Bonsdorff et al. 1997b). In fishes, for example, low levels of dissolved oxygen affect their locomotion, growth and reproduction and in severe cases may lead to large deaths (Jones and Reynolds 1999). However, the long term-effects of eutrophication on evolution and ecology of populations are poorly known (Candolin 2004). The few studies that considered the long-term effects of eutrophication demonstrate that the impacts may be severe. For example, Seehausen et al. (2007) demonstrated that visually impaired sexual selection due to turbidity caused a significant decline in species diversity of the cichlids in the Great Lakes of Africa.

Many coastal areas of the Baltic Sea are much affected by eutrophication. Nutrients coming in from the surrounding soil build up due to restricted water exchange, high temperatures, and complex topography (Bonsdorff et al. 2002). Sand gobies (Pomatochistus minutus) and three-spined sticklebacks (Gasterosteus aculeatus) are both species that breed in the shallow coastal habitats of the Baltic Sea. In early spring, males build nests and attract females through courtship activities to lay their eggs (Hesthagen 1977, Wootton 1976). Järvenpää and Lindström (2004) studied eutrophication-related alterations in sand goby mating systems. They found that mating success was more evenly distributed among males in turbid water (less skewed towards larger males) than in clear water. In other words, the opportunity for sexual selection and intensity of selection on at least body size was lower in turbid conditions. Turbidity also alters sexual selection in the three-spined stickleback via changes in visual signalling (Candolin et al. 2007, Engström-Öst and Candolin 2007, Heuschle and Candolin 2007, Wong et al. 2007, Candolin and Salesto 2006, Candolin 2004). In turbid water stickleback males were generally more active in courtship behaviour (Engström-
Öst and Candolin 2007), females needed more time and energy to find mates (Candolin et al. 2007), and the completion of nests was slower and male territories denser in a highly vegetated environment (Candolin and Salesto 2006, Candolin 2004). Wong et al. (2007) found that in the presence of male-male competition, stickleback males in poor condition courted females less and courtship was an honest indicator of condition. In clear water this difference in courting effort was much bigger than in turbid water. The honesty of the cue was hence reduced in turbid water. There is some evidence that compromises in visual mate choice may increase the importance of olfactory cues (Heuschele and Candolin 2007). In general, alterations in sexual selection strategies may have long-lasting consequences. When mate choice is compromised, offspring fitness can suffer and risk the future of the population (Wong et al. 2007).

1.4 Scope of this study

Female sticklebacks may choose their mates on the basis of olfactory or visual cues, and the intensity of olfactory vs. visual signals may vary according to the environment (Rafferty and Boughman 2006). Visual cues include the body size, and the intensity of male nuptial coloration and courtship behaviour (Nagel and Schluter 1998, Milinski and Bakker 1990, Bakker and Mundwiler 1994). Females are generally found to prefer brighter and bigger males (Barber et al. 2001). The use of olfactory cues is made possible by the MHC-gene complex (see above). Female sticklebacks are thought to be able to smell MHC-encoded peptides that are secreted from the males’ bodies (Milinski 2003), and this way to be able to tell whether the male has resistance towards certain parasites. This resistance may be inherited by the offspring. Stickleback females have been found to use odour as a sense to distinguish their conspecifics, to tell the difference between males and females, and even more, they can tell apart males who have nests and males that don’t (Rafferty and Boughman 2006, Häberli and Aeschlimann 2004).

The goal of this study was to find out whether the relative use of visual and olfactory cues in mate choice changes in the presence of turbidity, using the three-spined stickleback of the Baltic Sea as a model species. I also attempted to find out whether
these changes might influence the fitness of the population in the long run. The three-spined stickleback was a suitable model species for the study, because it is abundant in the Baltic Sea, and it is easy to catch and to handle. A lot is known about the ecology and behaviour of the three-spined stickleback, and its mating behaviour is very specific and easy to observe. There have been previous studies on the effect of turbidity on the behaviour of the fish in other respects (e.g. Wong et al. 2007, Candolin 2004), but the effect on the relative use of different mating cues has not yet been investigated.
2. Materials and methods

2.1. The study species

The three-spined stickleback is a small teleost and gets its name from the three dorsal spines anterior to the dorsal fin. Within the species three main morphological phenotypes are recognised based on how the armour-like lateral plates cover the sides of the body. The morphs may show different migration patterns (Bell and Foster 1994, Wootton 1984).

The distribution of the three-spined stickleback more or less follows the coasts of North America, Europe and parts of Asia (Orti et al. 1994, Wootton 1984). Latitudinally three-spined sticklebacks are found from 70 °N in Greenland and occur as far south as Japan and Korea (Wootton 1984). The populations are either resident in fresh water, marine or anadromous (they mostly spend their lives in the sea, but swim to fresh water to breed) (Orti et al. 1994). Freshwater populations are thought to have independently evolved from marine and anadromous forms, and they may differ from these in body structure (Bell and Foster 1994). In fresh water the fish may live in reproductively isolated benthic (bottom feeding) or limnetic (plankton feeding) populations (Foster et al. 2008).

Three-spined sticklebacks have good colour and form vision, which is suggested to be their main sensory modality. Olfaction may play an important role in reproduction, but seems to play little part in feeding. Three-spined sticklebacks are not known to have any form of sound reception (Wootton 1984). Mate choice in the three-spined sticklebacks potentially involves the use of both visual and olfactory cues (Bakker and Mundwiler 1994, Milinski and Bakker 1990, Milinski et al. 2005).

The breeding season of three-spined sticklebacks in the Baltic Sea population typically extends from May to late July. During the season, sex roles of males and females are clearly defined. Stickleback males obtain territories and build nests of substrate (sand, mud and algae) by gluing it together with a mucus string secreted from the kidneys. The nest composition may differ between individuals and is possibly genetically controlled
(Rushbrook et al. 2008). Stickleback males develop a carotenoid-based breeding pigmentation in the skin of their lower throat (Barber et al. 2001). There is individual and population based variation in the intensity of coloration (Wootton 1984). The majority of males seem to complete only one breeding cycle (Bakker and Mundwiler 1994). Stickleback females produce eggs at intervals of a few days, which take up a significant proportion of their body mass.

Males that have established territories and completed their nests attempt to attract females through a conspicuous courtship dance. The dance consists of several phases. When the male observes a gravid female in his territory, he approaches her with rapid zigzag-like motions, jumping from side to side towards the female. If the female shows interest, she responds by approaching the male in head-up position. The male turns and quickly swims to the nest and pokes the opening site in order to show it to the female. If the female decides to lay her eggs, the male creeps through the nest and fertilises them by releasing his sperm. After the fertilization the male stays in the proximity of the nest and applies quivering motions to increase the oxygen flow to the fertilised eggs, and guards the nest from predators (Wootton 1984).

2.2. Sample collection and maintenance

Wild adult three-spined sticklebacks were collected from Tvärminne Zoological Station from early May until mid-June, 2007. The fish were caught from the shallow waters of Långskär Bay (66.34 °N, 24.58 °E) in the proximity of the biological station by using minnow traps with a mesh size of 5 mm. The traps were undone every day, and the caught three-spined sticklebacks were brought back to the research station. Females and males were housed in separate flow-through aquaria in an outdoor facility under natural light conditions. The temperature in the tanks was raised and kept constant at 18 °C by underwater heaters, and artificial plants were placed in the aquaria to imitate natural conditions. Fish were fed frozen chironomid larva daily.
As soon as males started developing signs of nuptial colouration (their irises had turned blue and a hint of red colour had developed around their throats) they were transferred to individual 10 l-flow-through aquaria and supplied with a nesting dish (a plastic flower pot holder plate, 11 cm in diameter) covered with 1 cm of fine sand and some filamentous algae (*Cladophora glomerata*) fixed in place with gravel for nest building. To motivate nest making, a gravid female in a transparent Plexiglas cylinder, through which the male could see and smell the female, was placed in each individual male tank every day for 20 minutes. A male was considered to be ready to breed as soon as it had built a nest and crept through it at least once.

### 2.3. Preparation of turbid water

Two algal cultures were chosen to make turbid water: *Brachiononas submarina* and *Isocrysis sp.* *Brachiononas* is a marine alga that belongs to the family of Chlamydomonadales and *Isocrysis* is a type of microalga that is often used in aquaculture. Both species are relatively easy to grow in wide temperature range and their growth can be stimulated by light (McGowan 2004). 60 l-buckets were filled with filtered sea water, and a small amount of either algal culture was added to each bucket along with a table spoon of fertiliser. The tanks were aerated and lights were placed on top of the buckets to increase the speed of algal growth.

When the algae had grown such that the water in the tanks started turning greenish in colour, turbidity was measured using a nephelometric turbidity meter. A nephelometric turbidity meter measures the scatter of light through the sample. The principle is that the more small particles there are in a sample, the more light will reach the detector in contrast to a sample that has large particles. The turbidity is recorded in nephelometric turbidity unit (NTU). When the turbidity readings in the tanks reached around 30 NTU, which took a few days, the water was diluted with sea water to yield a turbidity of 6.1 +/- 1.3 NTU. In the Baltic Sea the turbidity has been generally found to range from 1–45 NTU in fish breeding areas (Granqvist and Mattila 2004). The turbidity of 6.1 was
chosen, because in higher readings, visibility decreases quickly and it is no longer possible to observe the fish.

2.4 Experimental setup

2.4.1 Choice experiments

Two Plexiglas-fluviarums (Figure 1) were built, one for the tests with turbid water and the other one for clear water. The fluviarums had three compartments: one for the female and two for males and their nests. The male-compartments were separated from each other with an opaque Plexiglas to prevent male-male interactions. Between the female compartment and the two male compartments, three different female/males-separation walls were built to be altered between the experimental runs:

(1) An opaque Plexiglas-wall with holes (to test for olfactory cues)
(2) A transparent Plexiglas-wall without holes (to test for visual cues)
(3) A transparent Plexiglas-wall with holes (to test for both olfactory and visual cues)

Water current with a speed of 1.67 ml s\(^{-1}\) was created to run through the fluviarums with the aid of a 10-l-tank with an adjustable hose. The tank was placed behind the fluvium. Prior to the start of the experiment, both the tank and the fluvium were filled with either clear (2.0 +/- 0.7 NTU) or turbid (6.1 +/- 1.3 NTU) water. In the tests for olfactory cues and both cues, water was allowed to flow out behind the nests creating a current towards the female so that she could smell both males equally. In the visual test, hoses were used to deliver the water flow past the male-compartments to the female’s side (Figures 1 and 2). In all of the three runs, the female thus experienced a flow of water, but in the visual run there were no male olfactory cues in the water flow.

Two females were subjected to the three experimental runs (olfactory cues, visual cues and both cues), with the order of the two first runs being alternated between replicates. Two males in mating condition were selected randomly and placed with their nests in
the two male-compartments of the fluviarums. The following day, water current corresponding to the cue to be tested was allowed to run 10 minutes prior to the start of the experiment. If the experiment was performed in turbid water, the female was allowed to adjust to turbid conditions 30 minutes before starting the preference test. The female was placed in the middle of the front compartment in an enclosed Plexiglas-cylinder with a diameter of 10 cm. She was allowed to observe both males for 5 minutes, after which she was carefully released. During 15 minutes the time that she spent in the proximity of each male was recorded. Only the time during which the female was responding to the male within a 5 cm preference zone (marked with a black marker on the bottom of the fluviarum) was recorded. The male in front of which she spent at least 50% of active time was considered to be the preferred male. 45 experimental runs were performed in clear water and 48 in turbid water. 55 male groups were used, male group referring to a pair of males tested for preference with two consecutive females.

After each experiment the males were removed one at a time and placed in a transparent Plexiglas-photographing box. The box (10 cm x 5 cm x 20 cm) was filled with water, and a sponge covered with black photographing fabric was placed inside it to hold the stickleback male in place during photographing. The box was placed behind a Canon EOS D350 and completely covered with the fabric to minimize lighting from behind the fish. In the camera settings, the aperture was 10.0, shutter speed 1/200, and ISO 200. The in-built flash along with a white balance was used in order to guarantee natural colour representation. Photographing was done immediately after the experiments so that the males would not have time to change colouration.

All fish were weighted and measured for further analysis. After this, the follow-up experiment to evaluate offspring fitness, in-vitro fertilizations and raising of the offspring were performed in cooperation with two other students from the research group, MSc Jan Heuschele and MSc Ulla Tuomainen.
Figure 1. A schematic drawing of the fluviarum (modified from Engström-Öst and Candolin 2007). L = length, W = width, H = height. Female preference (for male A or B) was measured as the active time the female spent in the preference zone, i.e. within 5 cm from the removable wall. The arrows indicate the water entry point for olfactory cues and both cues (a) and for visual cues (b).
Figure 2. Experimental setup of the preference tests. The arrows indicate the direction of water flow. In two flow-through fluviarums (one filled with turbid water and the other with clear water) two males in breeding condition with their nests were placed in the back compartments, and a gravid female in the front compartment. Preference was tested in three consecutive runs. (1) In the test with olfactory cues, a solid divider with holes was placed to separate male compartments from the female compartment, and scented water current was run from males’ to the female’s direction. (2) In the test for preference with olfactory cues, unscented water was run through the female’s compartment, and the divider was replaced by a solid and transparent Plexiglas. (3) The divider was replaced with a transparent one with holes. Two females were tested per one set of males, and the order of (1) and (2) was randomized.
2.4.2 In-vitro fertilizations and raising of offspring

The in-vitro fertilizations were done following the procedure of Barber et al. (2001). The egg clutches of the females tested were stripped into moistened watch glasses by gently pressing with fingers (in this way the eggs come out easily without harming the fish). The clutches were split in half, and each half was placed to a new watch glass to be artificially fertilized. One half was fertilized with the sperm of the preferred male and the other half with the sperm of the non-preferred male. In order to get the sperm from the males, they needed to be killed. This was accomplished by quickly cutting their head off with a pair of scissors directly behind their eyes. To obtain the gonadal tissue (the testes and the seminal vesicles), one incision was made from the vent horizontally along the body wall, and a second in an anterior direction at about 45° towards the dorsal spines. The body wall could then be folded back and the gonad could be removed intact. The same procedure was repeated on the other side to obtain the other gonad as well.

The gonads were carefully placed on a watch glass, and sperm was extracted by teasing them apart with fine needles. Sperm and eggs were gently mixed by agitating the watch glass, left for 15 minutes and transferred to incubators. Each clutch was then divided into two, giving two replicates of each full-sib family.

After fertilized, each clutch was rinsed with Methylene Blue to prevent moulding and placed in a flow-through tank in separate floating nets. In a few days, when eyes had appeared in developing eggs, the clutches were transferred to bigger holders so that they would have place to hatch. The eggs were removed one day before they were expected to hatch. After hatching the fish were fed daily with brine shrimp (*Artemia salina*). The fish fry were measured against a millimetre grid.
2.5 Data analysis

2.5.1 Effects of turbidity on relative use of cues

To analyse how turbidity influences the way how stickleback females use visual and olfactory cues relative to one another in mate choice, statistical tests were performed. A linear mixed effects (lme) model was plotted with software “R” version 2.5.1 (Pinheiro et al. 2007). Mixed-effect models, in comparison to linear models, include additional random effect-terms which makes them appropriate for data analysis with repeated measures (Fox 2002).

In the model, the cut off value was p>0.05, which means that values with p larger than 0.05 were automatically excluded from the analysis. Because two females were tested with the same pair of males, and each female was used in three consecutive runs, females were included as a random factor nested within male pairs. Interactions were calculated for all independent variables. If the interaction was significant, no other significances were taken into account. This means that the model started from the 3-way interaction and moved stepwise downwards (to 2-way interaction and to no interaction) until there was significance, if any was to be found. This type of analysis is called stepwise backward deletion.

In order to test how olfactory and visual cues were used in mate choice in clear and turbid water, a mixed model nested ANCOVA with the following variables was performed: the time of female with the left male in the presence of a single cue was a dependent factor, time of female with the left male in presence of both cues was a covariate, and water turbidity and type of cue were fixed factors. To measure the time with the left male, a relative measurement (time spent with the left male divided by the total time spent with the two males) was used. To find out if the order in which the cues were tested (visual cues first, or olfactory cues first) had an effect, order was included as a fixed factor in the initial mixed model nested ANCOVA.
To find out if females were more motivated to inspect mates in the presence of olfactory or visual cues, and to see if turbidity affects this, a mixed model nested ANOVA was used. Motivation refers to the time that females spent within 5 cm distance of the male during the experiment. The total time the female spent with the male was a dependent factor, and the type of cue (visual, olfactory or both) and water turbidity were fixed factors.

A post-hoc test (mixed model nested ANOVA) for the previous test was performed to find out whether the motivation of females in the presence of olfactory cues and visual cues was statistically significant between turbid and clear conditions. Here the time difference (time the female spent with the male in presence of olfactory cue minus the time spent in the presence of visual cue) was the dependent variable and water turbidity was a fixed factor.

To investigate if the male preferred by the female in the olfactory run was the same male that the female preferred in the visual run, a mixed model nested ANCOVA was used. The relative time spent with the olfactory cue was a dependent factor, the relative time spent with the visual cue was a covariate, and water turbidity was a fixed factor.

**2.5.2 Female preferences towards male traits**

The lme-model plotted in “R” was used to test female preferences toward male redness and length, separately.

In order to find out whether females preferred redder males, and if turbidity had an influence on this, a mixed model nested ANOVA was performed. The proportion of active time with the focal male (i.e. the left male which is the male used in the analysis) was a dependent factor, difference in redness between the males ((R_{male1} – R_{male2})/ (R_{male1} + R_{male2})) was a covariate, and water turbidity and cue type were fixed factors.
To obtain a value for the difference in redness, male redness was obtained the following way. The pigment colour of the fish was analyzed using an image analysis software Image J version 1.38w (http://rsb.info.nih.gov/ij/). The plug-in “Colour Threshold” (http://www.dentistry.bham.ac.uk/landinig/software/software.html) was used with the HSB colour system to select the red areas. The HSB (standing for hue, saturation and brightness) is the representation of points in an RGB colour space, which attempt to describe perceptual colour relationships more accurately than RGB, while remaining computationally simple. The values chosen were hue = 0–21 and 238–255, saturation = 83–255 and brightness = 0–255. The images were converted to an 8-bit gray-scale mode to give the extent of red pixels in the picture. The commands “Image/Adjust/Threshold” and “Analyze/Measure” were used to measure the area. The total fish area was first determined, after which the red area was measured and compared to the total fish area to give a percentage of redness.

In order to test if there was an effect of male length on female preferences, and to see how turbidity influenced this, a mixed model nested ANOVA was performed with proportion of time as a dependent factor, relative length difference between the males (\(\frac{\text{length}_{\text{focal}} - \text{length}_{\text{other}}}{\text{length}_{\text{focal}}}\)) as a covariate, and water turbidity and cue type as fixed factors. A post-hoc test to this test was also performed, in order to find out the direction of difference. In the post-hoc test proportional length difference was a fixed factor.

2.5.3 Offspring growth rate

The growth rate of 2444 fish fry, offspring from 80 males and 75 females, was analysed using a lme-model fit by REML. The statistical model used in the analysis was a mixed model ANCOVA, where the male running number (i.e. an individual number given for each fish) and female running number were random factors, treatment was a fixed factor, and male colouration, fish number and proportion of active time were covariates. P-values were calculated using a z-test on the estimates and standard errors.
3. Results

3.1. Effects of turbidity on relative use of cues

3.1.1 Relative use of cues

There was an effect of turbidity on the relative use of cues (mixed model nested ANCOVA, interaction between cue and turbidity: $F_{1,91} = 5.40$, $P = 0.022$) (Table 1). Visual cues were more important in clear water (stronger correlation) than in turbid water (Figure 3). The order in which the cues were tested had no effect (mixed model nested ANCOVA, interaction between cue type and turbidity: $F_{1,43} = 2.605$, $P = 0.114$).

Table 1. Results for the test of “relative use of cues” tested with mixed model nested ANCOVA plotted in the lme-model in “R”. The time of female with the left male in the presence of a single cue was a dependent factor, time of female with the left male in presence of both cues was the covariate ($T_{\text{both cues}}$), water turbidity and cue type were fixed factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimate</th>
<th>SE</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{both cues}}$</td>
<td>0.314</td>
<td>0.115</td>
<td>7.440</td>
<td>0.009</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.013</td>
<td>0.093</td>
<td>0.020</td>
<td>0.888</td>
</tr>
<tr>
<td>Cue type</td>
<td>-0.095</td>
<td>0.097</td>
<td>0.963</td>
<td>0.329</td>
</tr>
<tr>
<td>$T_{\text{both cues}}$  : Turbidity</td>
<td>0.149</td>
<td>0.153</td>
<td>0.939</td>
<td>0.338</td>
</tr>
<tr>
<td>$T_{\text{both cues}}$  : Cue type</td>
<td>0.241</td>
<td>0.160</td>
<td>0.665</td>
<td>0.417</td>
</tr>
<tr>
<td>Turbidity : Cue type</td>
<td>0.105</td>
<td>0.129</td>
<td>0.665</td>
<td>0.417</td>
</tr>
<tr>
<td>$T_{\text{both cues}}$  : Turbidity : Cue type</td>
<td>-0.496</td>
<td>0.214</td>
<td>5.404</td>
<td>0.022</td>
</tr>
</tbody>
</table>
3.1.2 Female activity

Both cue type and water turbidity influenced the total time that the female sticklebacks used to inspect the two males (mixed model nested ANOVA, $F_{2,187} = 5.59$, $P = 0.004$) (Table 2). Females were more active (used more time in inspecting) with visual cues than olfactory cues in clear water, but in turbid water there was no difference (Figure 4).

<table>
<thead>
<tr>
<th>Factor</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cue type</td>
<td>14.58 $^{2,187}$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.150 $^{1,49}$</td>
<td>0.701</td>
</tr>
<tr>
<td>Cue type : Turbidity</td>
<td>5.590 $^{2,187}$</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 2. The influence of cue type and turbidity on the female activity (total time that the female inspected the two males). Mixed model nested ANOVA with total active time as dependent factor and cue type and water turbidity as fixed factors.
3.1.3 Congruence in mate preferences

The male that the female preferred in the olfactory run was not the same male that she preferred in the visual run, thus females were not congruent in preferences (mixed model nested ANOVA: $F_{1,42} = 1.91, P = 0.17$). Water turbidity had no influence on this ($F_{1,42} = 0.029, P = 0.87$).

3.1.4 Female preferences to male traits

Redness difference between the two males did not have a significant influence on female preference (mixed model nested ANOVA: $F_{1,48} = 2.35, P = 0.13$). The redness difference was also independent of water turbidity (interaction between turbidity and redness difference: $F_{1,46} = 0.86, P = 0.36$) and of cue type (interaction between cue type
and difference in redness: $F_{2,183} = 0.29, P = 0.75$). However, female preference for male size depended on the type of cue (mixed model nested ANCOVA: interaction between cue type and relative length difference between the two males: $F_{2,187} = 3.60, P = 0.029$), but not on water turbidity ($F_{1,48} = 0.01, P = 0.93$). In the presence of visual cues, females preferred smaller males (mixed model nested ANOVA, $F_{1,49} = 4.37, P = 0.042$), but there was no effect of male length on female preference when olfactory cues (mixed model nested ANOVA, $F_{1,49} = 0.75, P = 0.391$) or both cues (mixed model nested ANOVA, $F_{1,49} = 0.039, P = 0.843$) were present (Table 3).

**Table 3.** Female preference for male length for each cue separately. Post-hoc test to the mixed model nested ANOVA with proportion of time as a dependent factor, relative length difference between the males as a covariate, and water turbidity and cue type as fixed factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Olfactory cues</th>
<th>Visual cues</th>
<th>Both cues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>342.3 $^{1,49}$ &lt;0.001</td>
<td>236.9 $^{1,49}$ &lt;0.001</td>
<td>201.4 $^{1,49}$ &lt;0.001</td>
</tr>
<tr>
<td>Length diff.</td>
<td>0.750 $^{1,49}$ 0.391</td>
<td>4.373 $^{1,49}$ 0.042</td>
<td>0.039 $^{1,49}$ 0.843</td>
</tr>
</tbody>
</table>

### 3.2 Offspring fitness effects

The redness of the male had a negative effect on the size of its offspring ($t = -2.350, P = 0.019$) and the number of fish per box correlated negatively with their size ($t = -33.42, P < 0.001$) (Table 4). Turbidity and the relative time that the female spent with the male had an influence on the size of the offspring ($t = 2.110, P = 0.035$) so that the relative time that the female spent with a male correlated positively with offspring size in turbid water, but negatively in clear water (Table 4 and Figure 5).
Table 4. Offspring growth results analysed with a mixed model ANCOVA, with female and male running numbers as random factors, treatment as a fixed factor, and male colouration, number of fish per box (No. Fish) and proportion of active time (T Active) as covariates. P-values were calculated using a z-test on the estimates and standard errors.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. error</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.510</td>
<td>0.028</td>
<td>55.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T Active</td>
<td>-0.021</td>
<td>0.017</td>
<td>-1.230</td>
<td>0.220</td>
</tr>
<tr>
<td>Treatment</td>
<td>-0.011</td>
<td>0.035</td>
<td>-0.320</td>
<td>0.750</td>
</tr>
<tr>
<td>Male colour</td>
<td>-0.260</td>
<td>0.110</td>
<td>-2.350</td>
<td>0.019</td>
</tr>
<tr>
<td>No. Fish</td>
<td>-0.023</td>
<td>0.001</td>
<td>-33.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T Active : Treatment</td>
<td>0.045</td>
<td>0.021</td>
<td>2.110</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Figure 5. The offspring length (cm) in relation to the relative time that the female spent with the male in presence of both cues.
4. Discussion

My goal was to study (1) whether increased turbidity due to eutrophication influences the relative use of visual and olfactory cues in the mate choice of the three-spined stickleback at the Baltic Sea population, and (2) whether these changes influence offspring fitness. This was accomplished by mate choice tests followed by raising artificially inseminated offspring. The main emphasis of my study was on the mate choice tests, because the sample size from collecting data over one stickleback reproductive season is limited for reliable testing of effects on offspring fitness.

4.1 Relative use of visual and olfactory cues in turbid water

Based on the mate choice tests turbidity influenced the relative use of visual and olfactory cues. Clear water produced a stronger correlation than turbid water between female active time with visual cue and female active time with both cues (Figure 3 A). In the olfaction-test, the correlation was stronger in turbid than in clear water (Figure 3 B). The strength of the correlation relates to the importance of the cue. In other words, visual cues are more important in clear water than in turbid water, and olfactory cues are slightly more important in turbid water than in clear water. In clear water the females were more active (i.e. they spent more time inspecting males) with visual cues than olfactory cues. In turbid water there was no difference (Figure 4).

Thus, as predicted, turbidity decreases the importance of visual cues in the mate choice of the Baltic Sea three-spined stickleback. The result agrees with other studies. Candolin (2004) found increased vegetation caused by eutrophication to reduce visibility and the rate at which stickleback females encountered mates. In her later study (2007) along with her colleagues she showed that the decrease in visibility in turbid water increases the time and energy that sticklebacks spend on courtship and mate choice and thus the costs of mating become larger in turbid environment. Increased mating costs gained more evidence from a study by Engström-Öst and Candolin (2007),
where the preferences of gravid stickleback females when tested by presenting them with a male who had a nest in turbid or clear water. It was found that males needed to court significantly more in turbid water to receive the same amount of attention from females as males in clear water, and that males in turbid water expressed more colouration than males in clear water. However, Engström-Öst and Candolin concluded that the final spawning decision was based on other than visual cues, possibly olfactory cues.

4.1.1 Visual cues

The compromised use of visual cues may have serious consequences to the population viability through impaired sexual selection. Visual cues sent by the stickleback males, particularly the courtship behaviour (including zigzag-like dance and other typically repeated behavioural patterns) and red nuptial colouration adopted during reproductive season, are known to be important in attracting and maintaining the attention of gravid females in mate choice (e.g. Candolin et al. 2007, Braithwaite and Barber 2000, Bakker and Milinski 1991, Milinski and Bakker 1992 and 1990, Barber et al. 2001, Kraak et al. 1999, McKinnon 1996, Bakker and Mundwiler 1994).

Males’ red colouration, and particularly its importance in mate assessment, has received much attention from researchers. Red colour indicates male quality in terms of their physical condition and parasite resistance (Milinski and Bakker 1990), as well as their parental abilities such as egg care behaviour (McKinnon 1996). Redness is also important in signalling males’ aggressiveness and capacity to win fights (Braithwaite and Barber 2000, Bakker and Mundwiler 1994). It is believed that the stickleback females use males’ redness in the final stage of mate choice to decide whether it is healthy and whether it has the right combination of MHC alleles to fight the current parasites (Milinski 2003, Aeschlimann et al. 2003).

Stickleback females usually prefer not only the brightest (Milinski and Bakker 1990), but also the largest males (Rowland 1989). Male size has also been found to correlate
with male health and better parasite resistance (Jäger et al. 2007, Kraak et al. 1999, Milinski and Bakker 1990). Both traits, redness and size, are honest indicators of condition (Zahavi 1975). It is possible that when visually based mate choice is limited (in this case due to reduced visibility caused by turbidity), females may not be able to choose the optimal male. This, in turn, may have long lasting consequences to population fitness. However, this depends on the adaptability of the population to the changing conditions.

There is some empirical evidence that relaxed sexual selection has lead to serious negative consequences for the viability of populations. In a study by Seehausen et al. (1997), turbidity caused impaired mate choice and decreased species diversity of the haplochromine cichlids of the African Great Lakes. The cichlids are known of their explosive evolution, and more than 500 different species have been reported. Within two decades, the species diversity declined dramatically. Seehausen and his colleagues showed that the increased turbidity was partly responsible for the decline. In cichlids males are usually brightly coloured from blue to red or yellow, and females are cryptic in coloration. Because mate choice in cichlids is at least partly visual, the ability to detect the right coloured mates maintains the diversity within and across species. When water becomes turbid, the entry of shortwave light is inhibited which narrows down the light spectrum. This interferes with the colour-based mate detection and leads to constrained mate choice. In the case of the African Great lakes, the impaired mate choice has lead to decrease in species diversity.

In the light of the results of this study, it appears possible that the viability of the three-spined stickleback population of the Baltic Sea may also be threatened by impaired sexual selection, and the mechanism may be somewhat similar to the African cichlids. Since it seems clear that visual cues play a key role in stickleback mate choice, as they do with the cichlids as well, it is very plausible that partly blocking their use will alter the typical mate choice process. While in the cichlids inhibiting the proper use of vision to judge male colour lead to not recognising your own co-specifics, in sticklebacks it may lead to loosing some information of mate condition.
Surprisingly, there was no effect of male redness on female preferences in the present study (mixed model nested ANOVA: $F_{1,48} = 2.35, P = 0.13$), not in turbid nor in clear water. This finding is somewhat surprising, because most studies have found an influence of male red colour on female choice (e.g. Braithwaite and Barber 2000, Bakker and Mundwiler 1994, Milinski and Bakker 1990, McKinnon 1996, McLennan and McPhail 1990, Rowland 1994).

However, there are some studies which found that females did not always select the reddest male (Ward and Fitzgerald 1987, Bakker and Milinski 1991, Milinski and Bakker 1992, McDonald et al. 1995). It has been suggested that such situations may arise when the differences in male sexual colouration are very small. This could result to the females ignoring the colour differences because they are not capable of making any distinction between duller and redder males, or females may choose to do so if other cues are more informative (Braithwaite and Barber 2000). The average difference in redness between the experimental males in our experiment was 0.0136 or 1.36%. The redness difference was calculated as $(A – B)/(A + B)$ where $A$ was the percentage of red area in the preferred male and $B$ the percentage of red area in the non-preferred male when both cues were allowed. It could thus be that the difference in redness was not large enough which could explain our result.

Typically in species with paternal care for the eggs larger males are preferred in mate choice, because they are of better paternal quality. For example, size can be the advantage to chase away intruders at the nest site and to win fights with other males. This has been found to be the case in some stickleback populations too (Kraak et al. 1999). Surprisingly in the present study, the opposite result was found. In the presence of visual cues, females preferred smaller males (mixed model nested ANOVA, $F_{1,49} = 4.37, P = 0.042$), but there was no effect of male length on female preference when olfactory cues (mixed model nested ANOVA, $F_{1,49} = 0.75, P = 0.391$) or both cues (mixed model nested ANOVA, $F_{1,49} = 0.039, P = 0.843$) were present (Table 3).

A possible explanation could be that since size is only one of the qualities of the male assessed by the females in mate choice, some other quality (for instance courtship
activity) could override. Sometimes a smaller male may even express more intensive courtship behaviour to gain female attention (personal observation). In a typical courtship ritual the male approaches the female in zig-zag-like pattern and swims back to the nest trying to show the nest opening to the female. It may also creep through the nest or stay near the nest opening site fanning. These rituals may occur several times and at different pace. An active male performs these actions several times and may also do this faster than a more passive male.

Some other factors that were not taken into account in this experiment were the nest quality and female condition, which also may have an effect on choice (Rushbrook et al. 2008, Bakker 1999, Kraak et al. 1999). For example, in the fifteen-spined stickleback (Spinachia spinachia) females prefer males with more secretional threads in their nests (Östlund-Nilsson 2001). This preference pattern has not been tested with other species of the Gasterosteidae-family, but there is a possibility it might extend to the three-spined stickleback as well. Female condition, in turn, may effect on mate choice if it is costly to be choosy (Bakker 1999).

### 4.1.2 Olfactory cues

Since the beginning of this decade MHC related cues in stickleback mate choice have received much interest (Rafferty and Boughman 2006, Milinski et al. 2005, Häberli and Aeschlimann 2004, Aeschlimann et al. 2003, Milinski 2003, Reusch et al. 2001). Currently it is believed that both visual and olfactory cues are essential in stickleback mate choice, and they may also be correlated with each other. For example, Jäger et al. (2007) found an association of male redness with one MHC class I allele.

In mate choice of many species there is an initial assessment based on long-range cues to choose a subset of mates. In this process several cues may be interacting, or may be used hierarchically to narrow down the search of mate. Choosing can also be active, so that the choosing sex visits several prospective mates, which leads to choice of one of them (Gibson and Langen 1996). In the case of sticklebacks, one presented scenario is
that females evaluate males at a distance according to the optimal number of MHC alleles and only upon seeing the male’s redness, decide whether it is healthy and whether it has the right combination of MHC alleles to fight the current parasites (Milinski 2003, Aeschlimann et al. 2003).

In the present study, olfactory cues increased in importance in turbid water, while the use of visual cues decreased. This finding agrees with what Heuschele and Candolin (2007) found in their experiments on the same species. They studied how the pH increase in eutrophied environment influences the stickleback mate choice cues in laboratory experiments. Increased photosynthesis in eutrophied waters reduces the amount of dissolved carbon hydroxide and thus augments the pH. Heuschele and Candolin tested how gravid stickleback females responded to male scent in a test aquarium divided in three compartments, where the female was placed in the middle. Male scented water was dropped in each side compartment, where the water inflow on one side had a normal (8.0) and the other side an increased (9.5) pH. The attraction of female was found to be higher towards the male scent in the higher pH compartment. Increased pH alone did not attract females more. According to the experimenters, a reason for this could be a change in the MHC peptide structure (due to higher pH) secreted by the male fish in mate choice process, which in turn may facilitate the binding or transportation of the scent cue to the olfactory receptors in the females. Also, a pH change could influence the chemical cues themselves (Brown et al. 2002). The results of this study supports these findings.

4.2 Offspring fitness effects

The redness of the male had a negative effect on the size of its offspring (t = −2.35, P = 0.019) (Table 4). In other words, the redder the father the smaller its offspring was. Barber et al. (2001) found that offspring of brighter stickleback males grew slowly and resisted parasitic infections better. They suggested that increased disease resistance may involve a cost in terms of early growth rate, which would explain the effect on size.
Our result of the decreased offspring size agrees with their result. It would have been interesting to see if the parasite resistance in our experiment also increased in the smaller offspring, but due to sample size limitation this test was not possible.

The finding of Barber et al. (2001) along with the findings of Milinski and Bakker (1990) conforms to the good genes-hypothesis according to which the ornament of the male, in this case his redness, reflects its good genetic quality (Møller and Alatalo 1999, Heywood 1989, Bell 1978, Zahavi 1975). By choosing a redder male the female passes on good genes, in this case the parasite resistance genes, to the offspring. This could be the case found in our experimental population as well, if parasite resistance was negatively correlated with size.

The number of fish per box correlated negatively with their size ($t = -33.42, P < 0.0001$), meaning that the denser the offspring had to grow the smaller they became (Table 4). This result is logical, since when the resources (e.g. nutrients in this case) become scarce for a population, there are not plenty of them for all individuals, which may decrease growth rate. In a dense population also stress may contribute to reduced growth rate.

Interestingly, the relative time that the female spent with a male correlated positively with offspring size in turbid water, but negatively in clear water (Table 4 and Figure 5). This may indicate that turbidity does influence the evolution of the population by altering offspring qualities. However, the sample size here was small, and these results should be interpreted with caution. More research is needed to determine how the population will cope in the long run. For example, offspring fitness evaluation is a good way to test this.
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