Mate choice in straight-nosed pipefish (*Nerophis ophidion*) may be affected by increasing turbidity in the Baltic Sea

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Contents

Abstract

Increasing turbidity in the Baltic Sea changes the visual environment for several fish species. This affects both sexual selection and mating behaviour for those species. In this study I tested if the sex-role reversed straight-nosed pipefish (*Nerophis ophidion*) is affected by increased turbidity. I did a mate choice experiment in both clear and turbid water, where the algae was added to water until it had similar turbidity to what the fish can encounter in the wild. Both mate choice and activity level was tested. I also did fecundity analysis on females from the clear water treatment in addition to test the correlation of secondary sexual traits. Fecundity of females was positively correlated with body length. The secondary sexual traits were also positively correlated with body length. In clear water the male chose the large female, in contrary to turbid water where the mate choice disappeared. Mate choice in the straight-nosed pipefish is negatively affected by the increased turbidity in the Baltic Sea, the same applied to activity level. This can cause a decrease in reproductive success for the male, and weaken the sexual selection on females.

Sammendrag

Økt turbiditet i Østersjøen forandrer det visuelle miljøet til flere fiskearter. Det påvirker både den seksuelle seleksjonen og paringsatferden for disse artene. I denne studien testet jeg om nålefisken liten havnål (*Nerophis ophdion*), som har reverserte kjønnsroller, blir påvirket av økt turbiditet. Jeg gjorde et partnervalgforsøk i klart og uklart vann, hvor alger ble tilsatt i vannet til uklarheten var lik det fisken kan møte i havet. Både partnervalg og aktivitetsnivå ble testet. Jeg gjorde også analyser på fekunditet og sekundære seksuelle trekk. Både fekunditeten og de sekundære seksuelle trekkene var positivt korrelert med kroppslengden til hunnen. I klart vann hadde hannen et tydelig partnervalg, mens dette forsvant i uklart vann. Partnervalg i liten havnål er påvirket negativt ved økt turbiditet i Østersjøen, og det samme gjaldt for aktivitetsnivået. Dette kan være med på å redusere den reproduktive suksessen til hannen og redusere den seksuelle seleksjonen til hunnene.

Introduction

Around the Swedish coastal areas eutrophication is one of main causes for alterations in ecosystems (Rönnbäck et al. 2007). Eutrophication is defined as an increased amount of dissolved particles in the water masses, which leads to increased growth of plants and algae and causes turbid water (Larsson et al. 1985).

In the Baltic Sea, the first sign of eutrophication in modern time occurred in the 1970s, where large amounts of filamentous algae were washed ashore along the coast (Rosenberg et al. 1990). The primary reason for increased eutrophication is more industry and fertilization used in agriculture (de Jonge et al. 2002). Larger phytoplankton blooms and more filamentous macroalgae are two effects of increased eutrophication (Larsson et al. 1985). With an increase in algae growth, there will be more sedimentation of organic matter, this may lead to anoxic condition and a decrease in the benthic fauna (Larsson et al. 1985).

One of the most productive coastal ecosystems is the seagrass meadows, which exists in every ocean from tropic to boreal regions (Duffy 2006). Seagrass meadows are under threats from eutrophication, overfishing and habitat destruction, which have caused regional extinctions (Duffy 2006). The increased primary production in the Baltic leads to negative effects for eelgrass (*Zostera marina*) in the seagrass meadows, as turbidity increases in the euphotic zone, and less light is available to photosynthesize (de Jonge et al. 2002, Wasmund and Uhlig 2003).

In aquatic environments high turbidity reduce the visibility of signals used during mate choice (Seehausen et al. 1997, Seehausen and van Alphen 1998, Candolin et al. 2007, Sundin et al. 2010), because increased amount of algae decreases the amount of light that penetrates the water column and therefore hampers the visual environment (Engström-Öst and Candolin 2007). This may lead to decreased mate choice, and weaken sexual selection (Candolin et al. 2007). Sexual selection was by Darwin (1871) defined as competition for the opposite sex and a choice of partners by the non-competing sex. During mate choice, different signals are assessed which have to be reliable (Johnstone 1996, Heuschele et al. 2009). Reliability of signals is accomplished by being costly to produce or display (Zahavi 1975, Kodric-Brown and Brown 1984).

Optical signals become practically one-coloured under water (Wiley 1994). Change in light absorption in turbid waters have an impact on the mate choice of multiple species in the Baltic Sea (Wong et al. 2012), where species that rely only on sight will have more difficulties to assess the quality of a potential partner (Seehausen et al. 1997, Rosenqvist and Berglund 2011). Turbidity may make it harder for a species to locate potential partners (Candolin and Voigt 2001). Reduced mate

encounters could decrease the choosiness of the choosy sex (Berglund 1995). A lower encounter rate of possible mates will probably weaken the sexual selection (Berglund 1995, Lindqvist et al. 2011).

It is not only mating ornaments that can be affected by increased turbidity also mating behaviour can be affected (Engström-Öst and Candolin 2007, Sundin et al. 2010). There can be a reduction in courtship behaviour, which can result in less mating because the activity need to be at a certain level before the mating can be accomplished (Candolin and Wong 2012), or a species may have to increase their courtship behaviour, which then could lead to increased predation risk (Engström-Öst and Candolin 2007).

In syngnathidae (pipefish, seadragons and seahorses) some species are sex-role reversed, which means that the male is the choosy one, and the females compete for the possibility to mate. When the operational sex ratio (OSR), number of males and females ready to mate, is female biased it can lead to sex role reversal (Vincent et al. 1992, Vincent et al. 1995). In the straight-nosed pipefish (*Nerophis ophidion*) the male receive eggs from only one female, and the female has capacity to produce more eggs than the male can carry. This leads to a strong sexual selection on the females because males are a limiting resource for the female. This results in choosy males and competing females (Berglund et al. 1986a, Rosenqvist 1990).

The broad-nosed pipefish (*Syngnathus typhle*) has been shown to be affected by increased turbidity. Males use longer time to assess the females (Sundin et al. 2010), and do not switch over to olfactory cues (Sundin et al. 2010, Lindqvist et al. 2011). Male gulf pipefish (*Syngnathus scovelli*) where able to locate females by chemical cues, but not differentiate on their size (Ratterman et al. 2009). One explanation for the ability of *S. scovelli* to locate females by chemical cues is probably because they live in dense seagrass meadows with high turbidity, which would select for other cues than vision (Ratterman et al. 2009).

Evidence of effects of turbidity has been found in other species as well. The three-spined sticklebacks (*Gasterosteus aculeatus*) rely on both visual and olfactory cues to assess possible mates (Heuschele et al. 2009). Heuschele et al. (2009) showed that female sticklebacks relayed more on visual cues in clear water than olfactory cues, but in turbid water they used both. Males in turbid water are only visible at short distances, which mean that they have to be more colourful and active during courtship (Engström-Öst and Candolin 2007). In clear water, female goodeid fish (*Xenotoca variatus*) preferred more speckled males, but this was not apparent in turbid water (Moyaho et al. 2004).

My aim is to investigate the impact of a turbid environment on mate choice and activity in *N.ophidion* in the Baltic Sea. If there is no mate choice in turbid water, there could be a reduction in

sexual selection on female because the secondary sexual traits are very important for mate choice in *N.ophidion* (Berglund et al. 1986a, Berglund et al. 1986b, Rosenqvist 1990). No mate choice in turbid water may reduce the fitness of the species, because the male brood fewer eggs or eggs with lower quality.

Hypothesis

Does turbidity affect mate choice and activity in straight-nosed pipefish?

Predictions:

- Males will choose mates randomly in turbid water
- The fish will be less active in turbid water

Materials and methods

Study species

Straight-nosed pipefish

The straight-nosed pipefish belong to the family Sygnathidae, which includes around 300 species (Wilson and Orr 2011). They occur in eelgrass meadows along the coast of the Mediterranean and the Atlantic (Moen and Svensen 2008), where they are quite cryptic (Vincent et al. 1995). Males brood the eggs openly on their abdomen, which takes between four to six weeks (depending on temperature) and then give birth to free living offspring (Berglund et al. 1986b). Female's secondary sexual traits are blue ornaments and a ventral skinfold (Berglund et al. 1986b, Rosenqvist 1990). The skinfold will increase the size of the females and are used in mate choice (Berglund et al. 1986b). Fecundity in *N.ophidion* on the Swedish west coast is positively correlated with the length of the female and the amount of blue colourings (Berglund et al. 1986a). Males receive their entire clutch of eggs from one female, and no correlation between length and fecundity has been found (Berglund et al. 1986a). Males are smaller than females, lack the secondary sexual traits, and the abdomen is flattened to incubate the eggs. When males has chosen a partner his snout turns yellow to indicate that he is willing to mate (Vincent et al. 1995).

Study area

The Baltic Sea (54°-66° N, 10° 30'-31° E) is a semi-closed sea, where the salinity decreases from west to east and south to north (Larsson et al. 1985, Rosenberg et al. 1990, Wasmund et al. 2001, Bonsdorff et al. 2002). It covers an area of 415 266 km^2 (HELCOM 2013), and its volume is 21 547 km^3 of water (HELCOM 2013). The average depth of the sea is 53 m (Bergström and Carlsson 1994, Wasmund et al. 2001, Rönnbäck et al. 2007, HELCOM 2013). The salinity in the Baltic varies, from marine condition (35 PSU,(Practical Salinity Units)) at Skagerak, and almost freshwater (1-2 PSU) in the Bothnian Bay (Rönnbäck et al. 2007, HELCOM 2013). The island Gotland lies in the middle of the Baltic Sea, where the salinity range between 6 PSU and 13 PSU (Rosenberg et al. 1990, Wasmund and Uhlig 2003). Eelgrass meadows around Gotland occur on sandy bottoms in depth between three to eight meters (Rönnbäck et al. 2007).

Fieldwork
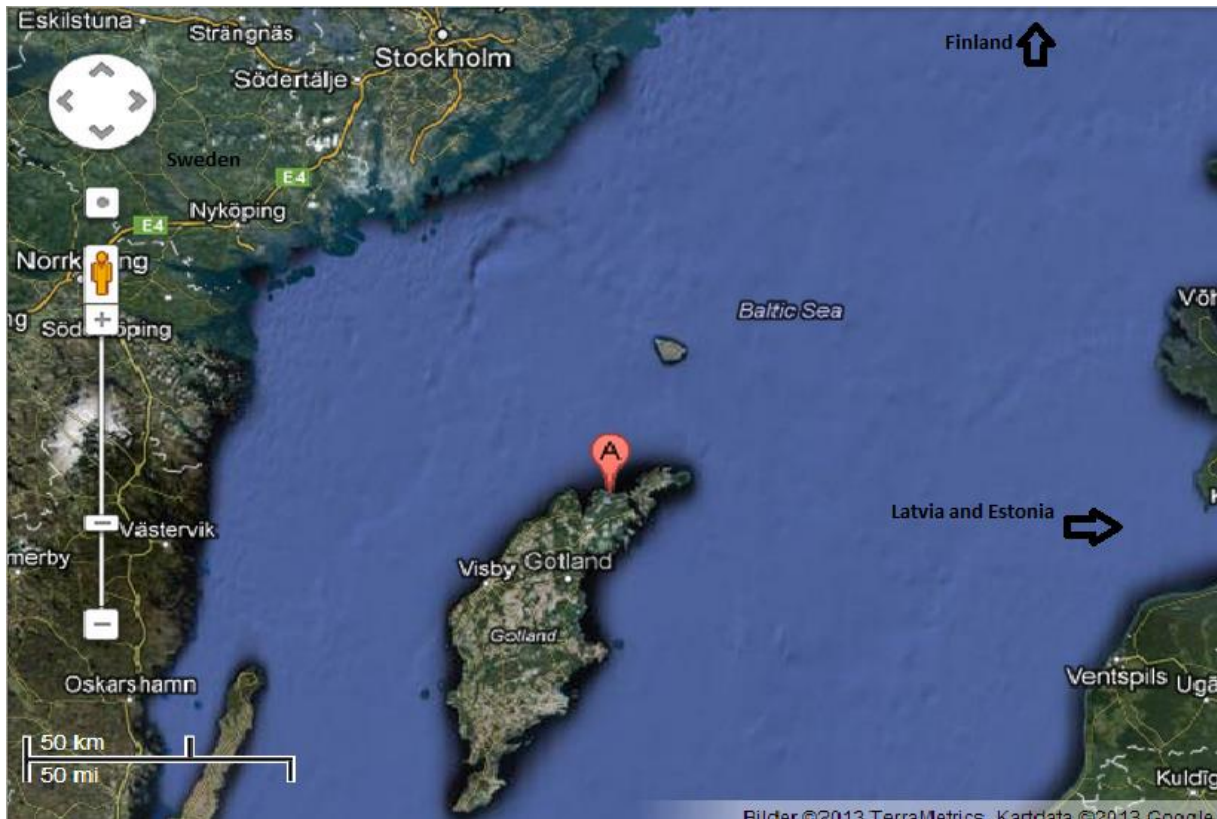
Figure 1: Map of Gotland and the Baltic Sea, Ar research station is marked with the A (Google Maps 2013).

The fish were caught by trawling with a small beam trawl with mesh-size 4 mm in eelgrass meadows. The trawl was pulled by a small boat through eelgrass meadows in Kyllaj (57° 34' N; 18° 57' E) on depths less than 10 m.

The fish were sorted into buckets based on species and sex. They were transported, live, in cooling bags with fresh sea water and extra supply of oxygen. Transportation took less than 20 minutes from the dock to the research station.

In the research station the fish were sorted once more and acclimatized to the water in small buckets in the 650 L tanks. The tanks had continuous flow of water and extra supply of oxygen. There was also fake eelgrass in the tanks so the fish could hide and rest int. The temperature in the tanks followed the natural conditions, which was between 9-14 °C. The salinity followed the natural conditions which varied between 6 PSU and 6.5 PSU. The tanks were cleaned twice a day, and the fish were fed ab lib frozen mysids, artemia and live artemia three times a day.

Mate choice experiment

To investigate how mate choice in *N.ophidion* would be affected by turbid water, mate choice was tested in both clear and turbid water.

The aquariums were divided into three compartments (fig.1). The females were separated from each other by a non-transparent wall, to hinder female-female competition, and the male was separated from the females by a transparent wall to avoid actual mating. All three compartments had fake eelgrass for the fish to hide and rest in. Each of the treatments had 25 replicates.
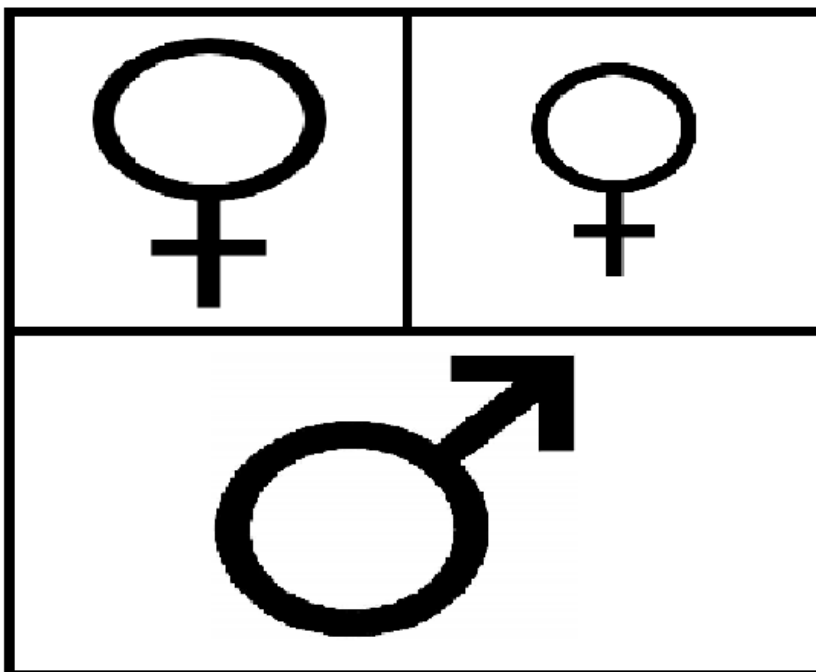
Figure 2: The setup of the aquariums to test for mate choice in *N.ophidion*. One large compartment for the male and two small compartments for the females, a transparent wall separated the male from the females. The male was considered to choose the female he was in front of at the time.

The water used in the experiment was between 14 °C to 16 °C to standardise between replicates. This was accomplished by pre-heating the water, both in the aquariums and large barrels, overnight. The barrels and aquariums had extra supply of oxygen, so the water would not have a shortage of oxygen after standing all night. The oxygen supply was taken out of the aquariums during the experiments. The fish were not fed during the experiment.

In the turbid treatment, water was prepared in barrels beforehand, giving approximately the same turbidity in each replicate. The mean measured turbidity was 5.41±0.014 NTU (Nephlometric Turbidity Units) in the male compartment, 5.70±0.144 NTU in the compartment of the large females,

and 5.42±0.129 NTU in the compartment of the small females (no sign of difference in female turbidity. ANOVA, p-value: 0.466). These values are similar to the turbidity the fish may encounter in the wild (Granqvist and Mattila 2004). I added instant algae mix (Nanno 3600, CCMP 525 Nannochlorpsis sp) to the large barrels and measured the turbidity by a portable turbidity meter (HANNA HI93703), and measured the turbidity again in each of the three compartments. The set up for clear and turbid treatments were the same, and all fish were used just once.

The females were selected after length, as the blue ornaments and skinfold is highly correlated to length (Berglund et al. 1986a). Males could choose between one large female and one small female in each of the replicates (fig. 1). Mean size of males used in the clear water treatment was 139.33±2.58 mm and 136.80±2.26 mm in the turbid water treatment, there was no significant difference between treatments (ANOVA, p-value: 0.464). Mean size of the large females were 210.47±2.37 mm in clear water and 204.48±2.63 mm in turbid water, there was no significant difference between treatments (ANOVA, p-value: 0.102). Mean size of the small females were 180.33±2.13 mm in clear water and 180.12±2.01 mm in turbid water, there was no significant difference between treatments (ANOVA, p-value: 0.942). All fish used were mature.

The large and small females were placed randomly on the two sides of the aquariums. The fish were acclimatized for 30 minutes, during this time males were visually separated from the females by a non-transparent wall. The non-transparent wall was removed and the replicates ran for three hours with scan sampling of the male every 10 minute, giving a total of 17 scan samplings per male. The position of the male was recorded as in front of the large female or the small female. Activity was noted as how often the male shifted side in the aquarium, from one female to the other female.

After each of the replicates the total length for both male and female, and skinfold length and width for the females were measured to the nearest half millimetre. The skinfold width was measured as width of the body and the skinfold combined.

The water was changed between each replicate, to hinder any residues from the last fish affecting behaviour of the new fish.

Ovaries are a good measurement on fecundity in fish (Coates 1988, Berglund et al. 1989, Rosenqvist 1990, Kjesbu et al. 1991) and to investigate how length is correlated to ovary size females in clear water were killed and dissected for their ovaries after the replicate was done. First they were sedated in a benzocaine solution before they were photographed, and measured. The fish were killed by decapitation before dissection. Ovaries were taken out by clipping up the abdomen from the anus and up. Ovaries are then visible as two orange strings inside the abdomen. They were carefully taken

out with a pincer and put on a piece of aluminium foil, marked with an individual number and date. The ovaries were dried in a drying cabinet for at least 24 hours in 70 °C. After drying, they were weighted on an analytic weight.

After the replicates were done, all live fish not used for fecundity analyses were put back into the tanks after acclimatization in small buckets.

Ethical permit

All handling of fish were done under the licence Dnr S 155-09 from the Swedish Board of Agriculture.

Statistical analysis

I used R2.15.3 (R Development Core Team 2013) to conduct all my statistics.

To test for difference between the two treatments in male and female length, skinfold length and skinfold width for females I did an analysis of variance (ANOVA). I also used ANOVA to test for difference in turbidity between the large and small female.

To calculate the correlation of skinfold length, skinfold width and dry weight of ovaries with female length as the explanatory variable, I used a linear model (LM). The plots were made with the plot-function in R. To test whether all three traits was correlated with each other I did a correlation test.

To test if the males chose their mate in clear water and turbid water I used a binomial test. The success (how often the males were in front of the large females) was tested against the total sample size (in front of both females). This was tested against a 0.5 probability.

To test for significant difference in choice (in front of the large female) between the two treatments I used a general linear model (GLM) with binomial errors. I used binomial errors because the data was proportion with success/failure, and to correct for over dispersion I used quasibinomial. To make the response variable I combined the two variables, in front of large female and in front of small female, with the cbind function. To back transform the estimates I used the ilogit function from the Faraway package (Faraway 2011).

To test if there was a significant difference in male activity between the two treatments I used a GLM, with negative binomial errors, because of count data. The data was over dispersed, therefore did I not use poission, but negative binomial errors. Negative binomial errors are a better fit than quasipoisson because it is a more general model. To do the negative binomial errors I used the negative binomial function from the mass package (Venables and Ripley 2002), with activity as response to treatment. To back transform the estimates, I used a log-function.

To do the graphs for both choice and activity, I used ggplot2 (Wickham 2009), together with plyr (Wickham 2011), plotrix (Lemon 2006), doBy (Højsgaard et al. 2012). Treatment is the explanatory variable, and the proportion of choice and the activity is the response variables.

Results

Mate choice

In the clear water treatment males chose the larger female (p<<0.001, 95% confidence interval: 0.63-0.72, binomial test). In the turbid water treatment males did not choose a female (p-value: 0.5606, 95% confidence interval: 0.47-0.56, binomial test) (fig. 3).

There is a biological difference in mate choice between the two treatments (GLM (binomial errors), p-value: 0.1433), where mate choice disappears in turbid water (fig. 3).
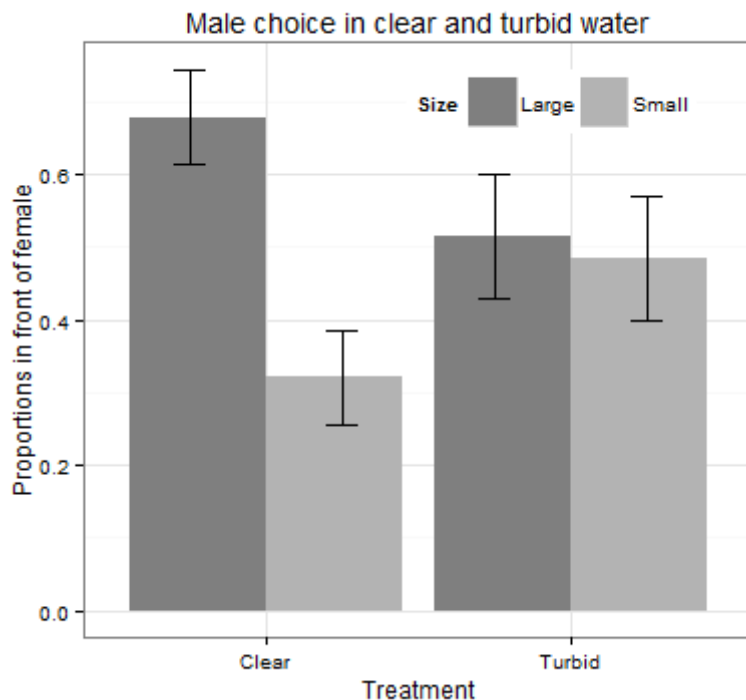
Figure 3: Proportion of time the male *N.ophidion* spent in front of large and small female in clear and turbid water treatments. Y-axis: proportion of time in front of females, x-axis: treatment. There was no significant difference between the treatments, (GLM (binomial). N in clear water: 24, estimated time spent in front of large female: 0.68±0.065, in front of small female: 0.32±0.065. N in turbid water: 25, estimated time spent in front of large female: 0.52±0.087, in front of small female: 0.48±0.087. P-value: 0.1433).

Fecundity

I found a significant correlation between weight of the ovaries and length of the female (p<<0.001). The weight of ovaries increases with increasing body length of females with a slope of $5.2*10^{-4}$ ($\pm 5.68*10^{-5}$) (fig. 4).
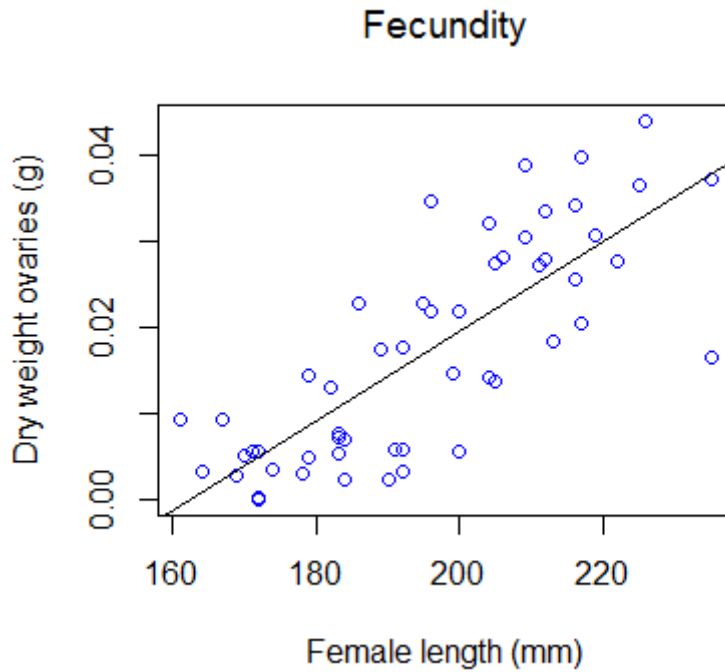
Figure 4: Correlation between length of female *N.ophidion* and ovary weight. Y-axis: dry weight of ovaries (g), x-axis: female length (mm). Dry weight ovaries $=-8.43*10^{-2}(\pm 1.11*10^{-2})+5.2*10^{-4}(\pm 5.68*10^{-5})*$female length. T-value=9.2, p<<0.001.

Secondary sexual characters

There is a significant correlation between body length of the female and skinfold length (p<<0.001).

Skinfold length increases with female body length with a slope of 0.599(±0.0295) (fig. 5).

Skinfold lenght (mm)

Figure 5: Correlation between length of female *N.ophidion* and length of the skinfold. Y-axis: skinfold length (mm), x-axis: female length (mm). Skinfold length = -29.17(±5.795)+0.599(±0.0295)*female length. T-value=20.3, p<<0.001.

It is a significant correlation between skinfold width and female body length (p<<0.001). Skinfold width increases with female body length with a slope of 0.0288(±0.0863) (fig.6).
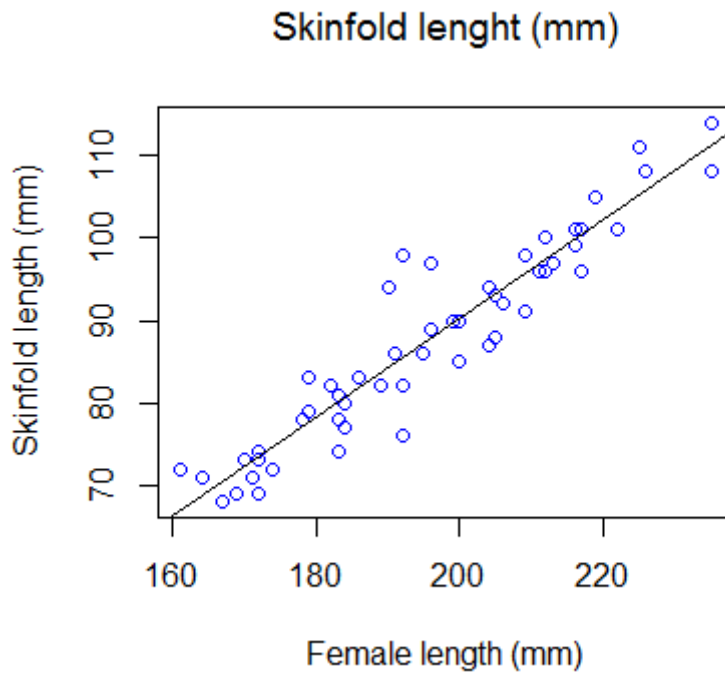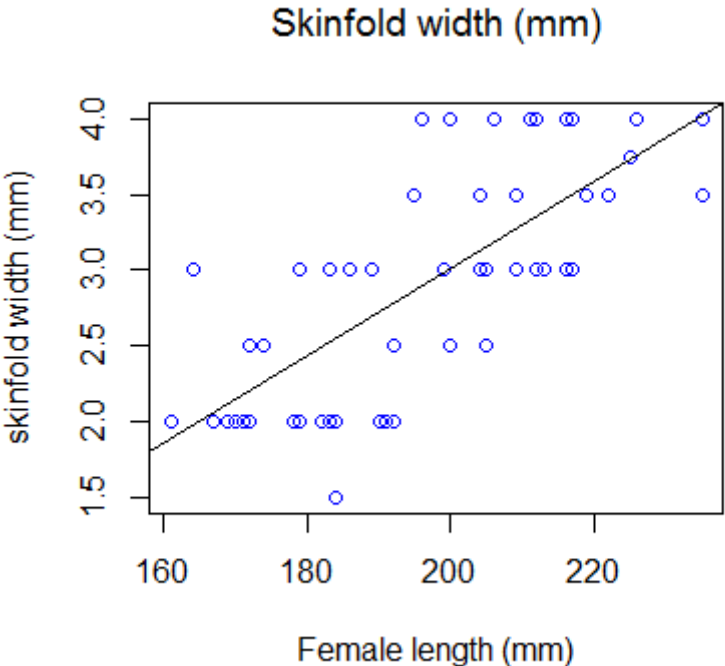

Skinfold width (mm)

Figure 6: Correlation between body length of female *N.ophidion* and skinfold width. Y-axis: skinfold width (mm), x-axis: female length (mm). Skinfold width = -2.76(±0.763)+0.0288(±0.0863)*female length. T-value=7.4, p<<0.001.

All three female traits, body length, skinfold length and skinfold width, are all correlated with each other (table 1). Body length and skinfold length have a positive correlation (cor-test: 0.94, p<<0.001), the same applies to body length and skinfold width (cor-test: 0.72, p<<0.001), and skinfold width and skinfold length (cor-test: 0.71, p<<0.001). The correlation between female body length and skinfold length is the strongest one, but there is still a strong positive correlation between the other two (table 1).

Table 1: The correlations between the three female (*N.ophidion*) traits (body length, skinfold length and skinfold width).

Traits	Degree of freedom	T-value	P-value	95 % - confidence interval	Correlation
length, skinfold length	51	20.3	$2.2*10^{-16}$	0.90-0.97	0.94
length, skinfold width	51	7.4	$1.15*10^{-9}$	0.56-0.83	0.72
skinfold width, skinfold length	51	7.2	$2.74*10^{-9}$	0.54-0.82	0.71

Activity

The male activity in clear and turbid water was significantly different from each other (GLM (negative binomial), N in clear water: 24, N in turbid water: 25, p-value: 0.0313) (fig. 7). Males in clear water shifted from one female to the other with a mean of three times (2.97±0.055), while males in turbid water had a significantly lower activity with a mean of one shift between females (1.28±0.258).

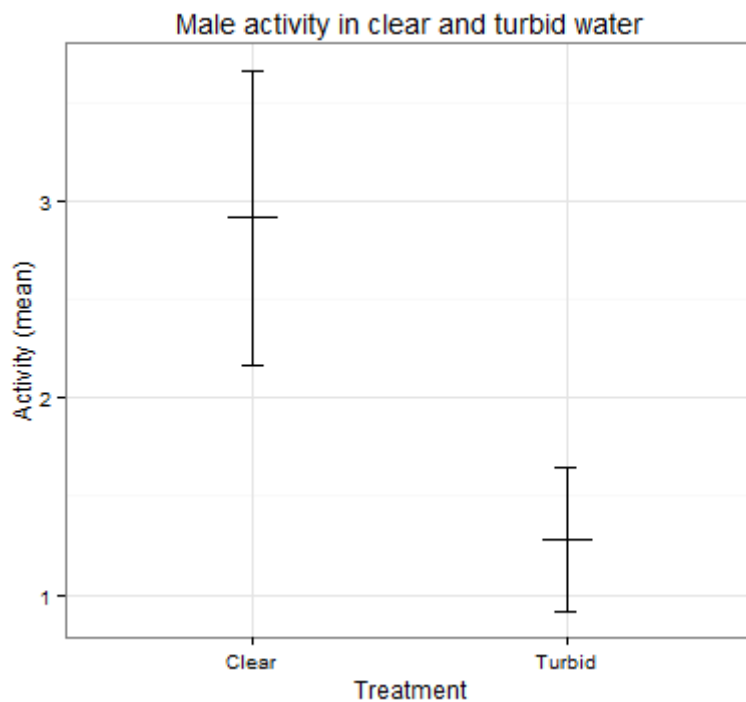**Figure 7: The mean activity of male (*N.ophidion*) in clear and turbid water. Y-axis: mean activity of males, x-axis: treatment, clear or turbid water. Activity was measured as shift from one female to the other. There was a significant difference between treatments (GLM (negative binomial), N in clear water: 24, with estimated activity: 2.97±0.055, N in turbid water: 25, with estimated activity: 1.28±0.258. P-value: 0.0313).**

Discussion

Anthropogenic disturbance causes rapid environmental changes in the sea (de Jonge et al. 2002). Increased turbidity has been a challenge for several species of fish around the world (Luyten and Liley 1985, Seehausen et al. 1997, Järvenpää and Lindström 2004, Moyaho et al. 2004, Engström-Öst and Candolin 2007, Sundin et al. 2010). The aim of this paper is to improve the knowledge of how anthropogenic disturbances can affect sexual selection and mating behaviours in fish.

Both mate choice and activity in male *N.ophidion* are affected by increased turbidity in the Baltic Sea. Males had a more random mate choice and lower activity in turbid environments, which could lead to decreased fitness for the males because of fewer eggs transferred from the females.

I found that *N.ophidion* males chooses the larger female in clear water in the Baltic Sea (fig.3), as they do on the Swedish West coast (Berglund et al. 1986a). In turbid water mate choice disappear, and are more randomized (fig. 3). There is a biological difference between treatments. Because males actually choose mates in clear water, but did not do so in turbid water. Findings of Järvenpää and Lindström (2004) in sand goby (*Pomatoschistus minutus*) are in accordance with my results, females did not choose mates based on size in turbid water. It may be that searching for a mate in turbid environments is so time consuming that males choose partners randomly, as to secure at least one mating (Heubel and Schlupp 2006). Not only size is important in mate choice of *N.ophidion*, the blue ornaments on the female are also important (Berglund et al. 1986b, Rosenqvist 1990). Males in my study may not have been able to assess the females blue ornaments due to the fact that blue colour is more scattered in turbid waters (Wiley 1994). Males could therefore have problems using multiple cues during the choice, and have no mate choice in turbid water.

Secondary sexual traits for the female are positively correlated with body length (fig. 5 and 6). If males have difficulties assessing the female size, both skinfold length and width can assist in mate choice. These three traits are positively correlated with each other (table 1), so males will have several signals to assess. Berglund et al. (1989) showed that fecundity of female *N.ophidion*, on the west coast of Sweden, increases with body length. My study show the same pattern, where females in the Baltic Sea have higher dry weight of ovaries when they are longer (fig. 4). Skinfold length and skinfold width are strongly correlated with female body length (table 1). This means that both of these traits could be used as measurement for female fecundity. Skinfold length, with a correlation of 0.94 to body length would be a better choice than skinfold width, because of a correlation with body length of 0.71 (table 1).

No mate choice in turbid water could lead to decreased reproductive success for the males, which was found in *S.typhle* in the Baltic Sea (Aronsen 2012). *N.ophidion* males would experience the same decrease in reproductive success as mating with smaller females give males fewer eggs, This is amplified as *N.ophidion* males only get eggs from one female at the time (Berglund et al. 1986a). Increased turbidity would decrease the ability for adaptive mate choice, and could therefore reduce population viability (Candolin et al. 2007).

According to Engström-Öst and Candolin (2007) three-spined stickleback males increased their activity effort in display and courting in turbid water, this could be a compensation for lowered vision. In my study male activity level decreased in turbid water in comparison to clear water. Female activity was not observed in turbid water treatment, but I expect an increase in activity as to attract attention of the males. As courtship activity increases females will have to spend more energy on maintaining the elevated activity level. More time spent on actively courting males may enhance the risk of predation (Engström-Öst and Candolin 2007). In guppy (*Poecilia reticulata*) courtship displays were reduced in turbid water, and males relayed more on other tactics for successful matings (e.g insemination) (Luyten and Liley 1985). *N.ophidion* will probably not shift to other tactics, because females transfer the eggs to a male during a complex and prolonged mating dance (Rosenqvist 1990).

Increased turbidity in the Baltic Sea changes the visual environment for the sex-role reversed pipefish *N.ophidion*. Turbidity negatively affected both mate choice and activity level in males. There was evident mate choice in clear water, but this disappeared in turbid water. Thus do humans indirectly, by increased turbidity, affect mate choice in *N.ophidion*. The absence of mate choice in turbid environments could possibly lead to altered sexual selection, since males do not make an adaptive mate choice. When males fail to choose the most fecund female, there will be a reduced reproductive success for the males. Males decreased their activity level in turbid water, but I expect that the females increased their courtship activity to attract the male's attention, as was showed in three-spined stickleback (Engström-Öst and Candolin 2007). Further work on this topic may include a test of males ability to assess the blue ornaments on females in turbid water, and if the blue colour is intensified in turbid water. Since mate choice is absent in turbid water, a potential decrease in fitness should be studied. Female activity levels should also be looked at, as activity may change in turbid water.

References

ARONSEN, T. 2012. *Demographic, environmental and evolutionary aspects of sexual selection.* Philosophiae Doctor, Norwegian University of Science and Technology. ISBN: 978-82-471-4054-3.

BERGLUND, A., ROSENQVIST, G. & SVENSSON, I. 1986a. Mate choice, fecundity and sexual dimorphism in two pipefish species (Sygnathidae). *Behavioral Ecology and Sociobiology,* 19**,** 301-307.

BERGLUND, A., ROSENQVIST, G. & SVENSSON, I. 1986b. Reverses sex roles and parental energry investment in zygotes of two pipefish (Sygnathidae) species. *Marine Ecology Progress Series,* 29**,** 209-215.

BERGLUND, A., ROSENQVIST, G. & SVENSSON, I. 1989. Reproductive success of females limited by males in two pipefish species. *American Naturalist,* 133**,** 506-516.

BERGLUND, A. 1995. Many mates make male pipefish choosy. *Behaviour,* 132**,** 213-218.

BERGSTRÖM, S. & CARLSSON, B. 1994. River runoff to the Baltic Sea 1950-1990. *Ambio,* 23**,** 280-287.

BONSDORFF, E., RONNBERG, C. & AARNIO, K. 2002. Some ecological properties in relation to eutrophication in the Baltic Sea. *Hydrobiologia,* 475**,** 371-377.

CANDOLIN, U. & VOIGT, H. R. 2001. Correlation between male size and territory quality: consequence of male competition or predation susceptibility? *Oikos,* 95**,** 225-230.

CANDOLIN, U., SALESTO, T. & EVERS, M. 2007. Changed environmental conditions weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology,* 20**,** 233-239.

CANDOLIN, U. & WONG, B. 2012. *Sexual selection in changing environments: consequences for individuals and populations*, Oxford University Press.

COATES, D. 1988. Length-dependent changes in egg size and fecudity in females, and brooded embryo size in males, of fork-tailed catfishes (Pisces, Ariidae) from the Sepik river, Papua-New-Guinea, with som implications for stock assessments. *Journal of Fish Biology,* 33**,** 455-464.

DARWIN, C. 1871. *The descent of man, and selevtion in relation to sex,* London, Murray.

DE JONGE, V. N., ELLIOTT, M. & ORIVE, E. 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. *Hydrobiologia,* 475**,** 1-19.

DUFFY, J. E. 2006. Biodiversity and the functioning of seagrass ecosystems. *Marine Ecology Progress Series,* 311**,** 233-250.

ENGSTRÖM-ÖST, J. & CANDOLIN, U. 2007. Human-induced water turbidity alters selection on sexual displays in sticklebacks. *Behavioral Ecology,* 18**,** 393-398.

FARAWAY, J. 2011. faraway: Functions and datasets for books by Julian Faraway. *R package version 1.0.5.*

GOOGLE MAPS. 2013. *Ar, Gotland, Sweden*. Google. https://maps.google.no/maps?hl=no&tab=wl.

GRANQVIST, M. & MATTILA, J. 2004. The effects of turbidity and light intensity on the consumption of mysids by juvenile perch (*Perca fluviatilis* L.). *Hydrobiologia,* 514**,** 93-101.

HELCOM. 2013. *The nature of the Baltic Sea*. http://www.helcom.fi/environment2/nature/en_GB/nature/.

HEUBEL, K. U. & SCHLUPP, I. 2006. Turbidity affects association behaviour in male *Poecilia latipinna*. *Journal of Fish Biology,* 68**,** 555-568.

HEUSCHELE, J., MANNERLA, M., GIENAPP, P. & CANDOLIN, U. 2009. Environment-dependent use of mate choice cues in sticklebacks. *Behavioral Ecology,* 20**,** 1223-1227.

HØJSGAARD, S., HALEKOH, U., ROBISON-COX, J., WRIGHT, K. & LEIDI, A. A. 2012. doBy: doBy - Groupwise summary statistics, general linear contrasts, population means (Least-squares-means), and other utilities. *R package version 4.5-5*.

JOHNSTONE, R. A. 1996. Multiple displays in animal communication: 'Backup signals' and 'multiple messages'. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 351**,** 329-338.

JÄRVENPÄÄ, M. & LINDSTRÖM, K. 2004. Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*. *Proceedings of the Royal Society B-Biological Sciences,* 271**,** 2361-2365.

KJESBU, O. S., KLUNGSØYR, J., KRYVI, H., WITTHAMES, P. R. & WALKER, M. G. 1991. Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body-compsition. *Canadian Journal of Fisheries and Aquatic Sciences,* 48**,** 2333-2343.

KODRIC-BROWN, A. & BROWN, J. H. 1984. Truth in advertising - The kinds of traits favores by sexual selection. *American Naturalist,* 124**,** 309-323.

LARSSON, U., ELMGREN, R. & WULFF, F. 1985. Eutrophication and the Baltic sea - causes and consequences. *Ambio,* 14**,** 9-14.

LEMON, J. 2006. Plotrix: a package in the red light district of R. *R-News,* 6**,** 8-12.

LINDQVIST, C., SUNDIN, J., BERGLUND, A. & ROSENQVIST, G. 2011. Male broad-nosed pipefish *Syngnathus typhle* do not locate females by smell. *Journal of Fish Biology,* 78**,** 1861-1867.

LUYTEN, P. H. & LILEY, N. R. 1985. Geographid variation in the sexual behaviour of the Guppy, *Poecilia reticulata* (Peters). *Behaviour,* 95**,** 164-179.

MOEN, F. E. & SVENSEN, E. 2008. *Dyrelivet i havet, Nordeuropeisk marin fauna*, KOM forlag.

MOYAHO, A., GARCIA, C. M. & ÁVILA-LUNA, E. 2004. Mate choice and visibility in the expression of a sexually dimorphic trait in a goodeid fish (*Xenotoca variatus*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 82**,** 1917-1922.

R DEVELOPMENT CORE TEAM 2013. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna Austria*.

RATTERMAN, N. L., ROSENTHAL, G. G. & JONES, A. G. 2009. Sex recognition via chemical cues in the sex-role-reversed Gulf Pipefish (*Syngnathus scovelli*). *Ethology,* 115**,** 339-346.

ROSENBERG, R., ELMGREN, R., FLEISCHER, S., JONSSON, P., PERSSON, G. & DAHLIN, H. 1990. Marine eutrophication case-studies in Sweden. *Ambio,* 19**,** 102-108.

ROSENQVIST, G. 1990. Male mate choice and female female competition for mates in the pipefish *Nerophis ophidion*. *Animal Behaviour,* 39**,** 1110-1115.

ROSENQVIST, G. & BERGLUND, A. 2011. Sexual signals and mating patterns in Syngnathidae. *Journal of Fish Biology,* 78**,** 1647-1661.

RÖNNBÄCK, P., KAUTSKY, N., PIHL, L., TROELL, M., SÖERQVIST, T. & WENNHAGE, H. 2007. Ecosystem goods and services from Swedish coastal habitats: Identification, valuation, and implications of ecosystem shifts. *Ambio,* 36**,** 534-544.

SEEHAUSEN, O. & VAN ALPHEN, J. J. M. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behavioral Ecology and Sociobiology,* 42**,** 1-8.

SEEHAUSEN, O., VAN ALPHEN, J. J. M. & WITTE, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science,* 277**,** 1808-1811.

SUNDIN, J., BERGLUND, A. & ROSENQVIST, G. 2010. Turbidity hampers mate choice in a pipefish. *Ethology,* 116**,** 713-721.

VENABLES, W. N. & RIPLEY, B. D. 2002. Modern applied statistics with S. *Fourth Edition. Springer, New York*.

VINCENT, A., AHNESJÖ, I., BERGLUND, A. & ROSENQVIST, G. 1992. Pipefishes and seahorses: are they all sex role reversed? *Trends in Ecology & Evolution,* 7**,** 237-241.

VINCENT, A. C. J., BERGLUND, A. & AHNESJÖ, I. 1995. Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes,* 44**,** 347-361.

WASMUND, N., ANDRUSHAITIS, A., LYSIAK-PASTUSZAK, E., MULLER-KARULIS, B., NAUSCH, G., NEUMANN, T., OJAVEER, H., OLENINA, I., POSTEL, L. & WITEK, Z. 2001. Trophic status of the south-eastern Baltic Sea: A comparison of coastal and open areas. *Estuarine Coastal and Shelf Science,* 53**,** 849-864.

WASMUND, N. & UHLIG, S. 2003. Phytoplankton trends in the Baltic Sea. *Ices Journal of Marine Science,* 60**,** 177-186.

WICKHAM, H. 2009. ggplot2: elegant graphics for data analysis. *Springer, New York*.

WICKHAM, H. 2011. The split-apply-combine strategy for data analysis. *Journal of Statistical Software,* 40**,** 1-29.

WILEY, R. H. 1994. *Errors, exaggeration, and deception in animal communication*.

WILSON, A. B. & ORR, J. W. 2011. The evolutionary origins of Syngnathidae: pipefishes and seahorses. *Journal of Fish Biology,* 78**,** 1603-1623.

WONG, B. B. M., TUOMAINEN, U. & CANDOLIN, U. 2012. Algal blooms impact the quality of nest construction in three-spined sticklebacks. *Animal Behaviour,* 84**,** 1541-1545.

ZAHAVI, A. 1975. Mate selection - selection for a handicap. *Journal of Theoretical Biology,* 53**,** 205-214.