Mate choice, temperature, parasites and activity in a Baltic population of the broad-nosed pipefish *Syngnathus typhle.*

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Abstract

Potential reproductive rates (PRR) can be influenced by external factors like temperature and food availability, which in turn affects the operational sex ratio. This can make a population more biased towards one sex which influences how choosy individuals of the different sexes are.

In this study I allowed a focal individual of the broad-nosed pipefish *Syngnathus typhle* (either male or female) from a Baltic Sea population to choose between a large and a small stimulus fish of the other sex, in cold, intermediate tempered or warm water. I noted the activity of the focal fish in the different temperature treatments, which stimulus fish it spent most time in front of, and if the fish discriminated between parasitized and un-parasitized potential mates.

It has formerly been shown that the difference between male and female potential reproductive rates in *S. typhle* gets larger in colder water, i.e., the reproductive rate of the males slows down more than that of the females as temperature drops. Thus I predicted males to be choosier in cold water, and females to be choosier in warm water. I predicted the fish to be more active and to court more in higher temperatures and I also predicted the fish to discriminate against potential mates that are parasitized.

Parasitized fish were significantly larger than un-parasitized fish, although this might be a consequence of the larger fish being older in general and thus having been exposed to parasites for a longer period of time. There was a non-significant trend for males to prefer the un-parasitized fish. In contrast to the west coast population of *S. typhle,* larger mates were not preferred over smaller in any temperature, apart from that females in cold water had a tendency to prefer larger males. The focal fish were significantly more active in warmer water, independent of sex. They also courted more in warmer water. Males and females courted equally much, which indicates that the sex roles in the Baltic population are more equal than in the west coast population.

Introduction

The term "sexual selection" was first used by Darwin (1871) to explain why some individuals have characters and ornaments – secondary sexual characters – such as greater size, long tails, song, bright colouration or antlers, which cannot be explained by natural selection. In his book "The Descent of Man, and Selection in Relation to Sex" (1871), Darwin concluded, on the basis of his own observations and the observations of others, that males generally are more eager to mate – are having more "passions" – than females. He found that males more often fight among each other for access to females (male-male competition), and more often are the ones seeking for females to mate with. Males thus generally have more pronounced secondary sexual characters than females have (Darwin 1871).

Females, on the other hand, were described as generally more passive and "coy" in the mating, often even trying to avoid the eager males. He described how females often have to be courted in order to be willing to mate. Darwin also noted that the females seem to be doing some kind of choice and speculated that they might gain by choosing the most vigorous, healthy and spectacular males to mate with so that their offspring will inherit good male qualities, and in turn get many offspring (Darwin 1871).

Sexual selection most often operates more strongly in the males (Darwin 1871), but why is it this way? Why is it so common for males to have these secondary sexual characters, and so uncommon for females? How can it be that mainly males engage in sometimes lethal contests over matings, and that mainly males keep "harems" of two or more females? And how come it is mainly the males that invest valuable resources in producing greater body size, colourful or in other ways handicapping or spectacular ornaments? Why is it, among birds, males that have the most virtuous songs?

The answer, or at least part of the answer, can be found in the female and male gametes, and how much time and energy males and females invest in their offspring, respectively. The system of two sexes with one having large gametes and one having small gametes is called anisogamy. The female gamete, the egg, is always per definition significantly larger than the sperm, so that in each individual offspring the female gamete contributes both DNA and nourishment, while the male gamete contributes nothing more than DNA (Trivers 1972, Krebs et al. 1993, Andersson 1994). Females produce few and large gametes, while males produce numerous small gametes, and usually the former are significantly more costly to produce (Trivers 1972, Krebs et al. 1993, Andersson 1994). However, in species with much sperm competition males have been found to invest many resources into their testes, while in species where other forms of competition or no competition is the common pattern, the testes are smaller and the sperm less numerous (Stockley et al. 1997). One species where the male have small testes is the pipefish *Syngnathus typhle*, where by definition no sperm competition occurs (Kvarnemo and Simmons 2004), because males fertilize the eggs inside his own body (Berglund and Rosenqvist 2003). Small testes and a paternity confidence of 100% are probably true for the whole family of Syngnathidae (McCoy et al. 2001, Berglund and Rosenqvist 2003, Kvarnemo and Simmons 2004). An example of a species where some individuals have very large testes is the ruff (a lekking shorebird), where some males, called

faeders, mimic females. They probably get very few matings on the lek, but as faeders have 2.5 times larger testes than the dominant males (Piersma and Jukema 2006), they should have a good fertilizing success. As the dominant males cannot afford to both defend a lek and to have large testes, there must be some cost to sperm production.

In mammals, where internal fertilization is the pattern, females are physically bound to invest more time and energy, not only in the gamete production but also in nourishing and caring for the offspring inside their own bodies, and also continue the care even after the delivery. Mammalian males, on the other hand, usually gain more by deserting the female and searching for other females to mate with (Trivers 1972, Daly 1979, Krebs et al. 1993). Exceptions may occur if the females put demands on the males to be monogamous and stay and help to care for the offspring. Males may also stay if mate-guarding is necessary, and if females are hard to find. Then it might be better for the male to "guard what he has" and secure that the female will really give birth to his own offspring, rather than to go off trying to find another female that probably will be guarded by another male anyway. It might also be that the offspring actually needs two parents in order to survive.

In birds the care is often bi-parental, but it happens that only one parent – either the male or the female – cares for the offspring. Females still invest most heavily in the production of the gametes, and thus most often the males' potential reproductive rate (PRR, the reproductive rate when mate availability is not limiting) exceeds that of the females'. Exceptions occur, however, where the females are fast egg-layers and the male alone cares for the brood. Then, female PRR may exceed that of the males and a good female can thus monopolize many males. In species or populations where females compete more intensely over access to males than males compete over access to females, the sex-roles are said to be reversed. In birds this occurs for example in several jacana species (Jenni and Collier 1972, Emlen et al. 1998, Butchart 2000) and in the shorebird *Phalaropus tricolor* (Delehanty et al. 1998).

In insects and other invertebrates, parental care is less common. Thus the females, who still have to produce eggs with enough nourishment for the offspring to survive, invest more in the offspring and have a lower PRR than the males. The operational sex ratio (i.e. the number of males in proportion to the number of females in a population that are available for mating at any given time) thus becomes male biased, and males will generally compete more intensely for access to females and females may be choosier. Like in birds, there are several examples of sex-role reversal in insects. For instance, the males of the mormon cricket *Anabrus simplex* produces a large spermatophore which he offers to the female during courtship (Gwynne 1981). If she accepts it, he can mate with her while she feeds on it. The spermatophore is very costly to produce, and thus in this species the females compete more for access to mates when food is scarce because then they depend on the spermatophore for food. Males, when given the choice, prefer larger and more fecund females to mate with (Gwynne 1981).

In fish, no parental care is a common pattern, but when care occurs male care is more common. However, males may still have a higher PRR than females if they can fertilize and guard the eggs of several females simultaneously. For example, a male of the threespined

stickleback *Gasterosteus aculeatus* builds a nest to which he invites females to lay their eggs. Darwin mentions the same species in his book (1871), but under the synonym *Gasterosteus leiurus*. He writes that the male has been described as "mad with delight" when he encounters a female and that he tries to encourage and even force her to enter the nest. In addition to this courtship dance and harassment, the male has a bright red ornament on the belly, and bright blue-green eyes with which he tries to attract the females (Darwin 1871). This species thus exemplifies that male care does not necessarily lead to reversed sex roles.

In the family Syngnathidae one of the most extreme forms of male parental care occurs. The family consists of pipefishes, seadragons and seahorses, all sharing the unique character of male pregnancy (Wilson and Stolting 2007). The female transfers the eggs via an ovipositor (penis) into the male's brood pouch (i.e. *Syngnathus* and *Hippocampus*) or glue them directly onto his body (i.e. *Nerophis*) (Vincent 1992, Masonjones and Lewis 2000, Wilson et al. 2003). Thereafter the male protects and nourishes the eggs for several weeks – the length of the pregnancy differs depending on species (Berglund et al. 1989, Ahnesjö 1992, Yanagisawa and Matsumoto 2001, Scobell et al. 2009) and on the temperature of the water (*Syngnathus typhle*; Ahnesjö 1995, 2008). In the syngnathids, the sex roles can be either conventional or reversed, depending on the species (Vincent et al. 1992), though it may also differ spatially among populations within the same species, and temporally within populations (i.e. it may change during the breeding season; Kvarnemo and Ahnesjö 1996).

In the Swedish west coast population of the broad-nosed pipefish, *S. typhle*, females compete more vigorously over access to males than males compete over access to females, and males are the choosier sex (Berglund and Rosenqvist 1993). Thus, the sex roles in this population are reversed, although males can also compete over access to females and females can be choosy. The terms "conventional" and "reversed" sex roles seem to be less of a dichotomy than a gliding scale (Rosenqvist and Berglund 2011). Both sexes of *S. typhle* have been found to prefer a larger mating partner when given a choice (Berglund et al. 1986), and both have been found to compete over access to mating partners when put in a situation where the other sex is in minority. Males benefit by choosing larger females, because such females have more and larger eggs, and females benefit by choosing larger males, because they can provide more nourishment to each offspring, and also they can brood more offspring in the brood-pouch (Berglund et al. 1986). Parasites have also been found to play a role in mate choice, and *S. typhle* males preferred females with fewer parasites (Rosenqvist and Johansson 1995). Most often, however, females do not have the opportunity to choose because the operational sex ratio is female-biased. This, in turn, is because the PRR is higher in females than in males. Both females and males reproduce continuously throughout the reproductive season, but females have the potential to re-mate faster than males, and most females, especially the larger ones, have enough eggs to fill more than one male (Berglund et al. 1989, Berglund and Rosenqvist 1990). In cold water the males are really slow to re-mate because their embryos take longer to develop. In warmer water this difference in PRR decreases because the offspring then hatch faster (Ahnesjö 1995). Thus, male PRR significantly speeds up in warmer water, while female PRR is not so much affected. This makes huge differences in the operational sex ratio depending on temperature, and that should affect the sex roles as well.

The Baltic Sea population of *S. typhle* is not as thoroughly studied as the population on the Swedish west coast. What we know about the Baltic population is that they are smaller in size than the West coast population, which is usually the case with marine populations in brackish water (Bernes 2005). We also know that males of the Baltic population do not discriminate between males and females by smell only (Lindqvist et al. 2011), and that both the pipefish species in the Baltic Sea prefer eel-grass that is free from filamentous algae (Sundin et al. 2011).

In the experiment reported here, I investigated how temperature affects mate choice in *S. typhle* (i.e. big mate vs. small mate). I also checked how the activity (i.e. any kind of active movement made by the fish) and the amount of courtship were affected by temperature. I predict that males will be choosier in cold water, as their PRR then will be much lower than the female PRR. Females, on the other hand, are predicted to be choosier in warm water than in cold water, thus giving females more mating opportunities as the operational sex ratio will be less female biased. Activity, as well as amount of courtship, is predicted to be higher in warmer water, as it has been demonstrated that metabolic rate in fish are lower in lower temperatures (Lewis and Driedzic 2007). Discrimination against individuals infected with *Cryptocotyle spp.* and/or an unknown disease clouding one or both of the eyes, was investigated to see if choosiness regarding this differs between the Baltic Sea population and the population on the Swedish west coast, the latter investigated by Rosenqvist and Johansson (1995) who found that male *S. typhle* had a preference for females that were not infected with *Cryptocotyle spp*. I predict that both female and male pipefish will discriminate against infected individuals as that would be in accordance to Rosenqvist and Johansson's study (1995).

Material and methods
I did the experiment at Ar research station in northern Gotland, Sweden, in June 2011. The fish, *Syngnathus typhle,* were caught in shallow eelgrass meadows in Kyllaj, Gotland (57˚ 44' N; 18˚ 57' E) using a small motorboat dragging a beam-trawl (mesh-size 4 mm). Most of the fish were caught before the onset of the breeding season – 9th of May to 5th of June. The first pregnant male was caught the 4th of June. After capture, the fish were transported to the research station where they were kept in 650 l tanks, segregated by sex and, for most females, also by size in the categories "<130mm", "131mm-140mm", "141mm-150mm", ">150mm". The tanks were cleaned daily and the seawater was continuously renewed and oxygenated. The fish were provided with plastic eelgrass for shelter. Temperature and salinity followed the natural conditions (temperature 7-18˚C, salinity 6.3-7.1 $^o/_{oo}$). Natural and artificial (17L: 7D) light was used. The fish were fed with wild caught *Mysis spp.* and *Neomysis spp.* and frozen and laboratory-hatched *Artemia* spp. three times a day.

The experiment, designed to investigate if temperature affects mate choice and activity in male and female *S. typhle,* was carried out between 13th and 30th of June 2011.
I had six different treatments consisting of three temperatures with either males or females choosing partners: the cold treatment, the intermediate tempered treatment, and the warm

treatment. Each used either a focal female choosing between a male larger than herself, and a male smaller than herself, or a focal male choosing between a female larger or smaller than himself. The difference between the large and the small stimuli fish was 10 mm or more. The temperature was measured in the aquaria two times – once before the onset of the replicate, and once directly after the ending of it.

I used 140 l aquaria (40 cm x 50 cm x 70 cm) that were divided into 3 compartments; one large compartment for the focal fish (50 x 22.5 cm) and two small compartments (25 x 17.5 cm) for the stimuli fish. The barrier between the focal and the stimuli fish was a transparent plastic divider. The barrier between the two stimuli fish was a non-transparent plastic divider. In each corner of the aquaria, air-stones oxygenated the water. In each stimuli compartment one plastic eelgrass plant was provided for shelter, and in the focal compartment two plastic eelgrass plants were provided – one in front of each stimuli-compartment (Figure 1). Over the aquaria a 20 W halogen lamp provided light.
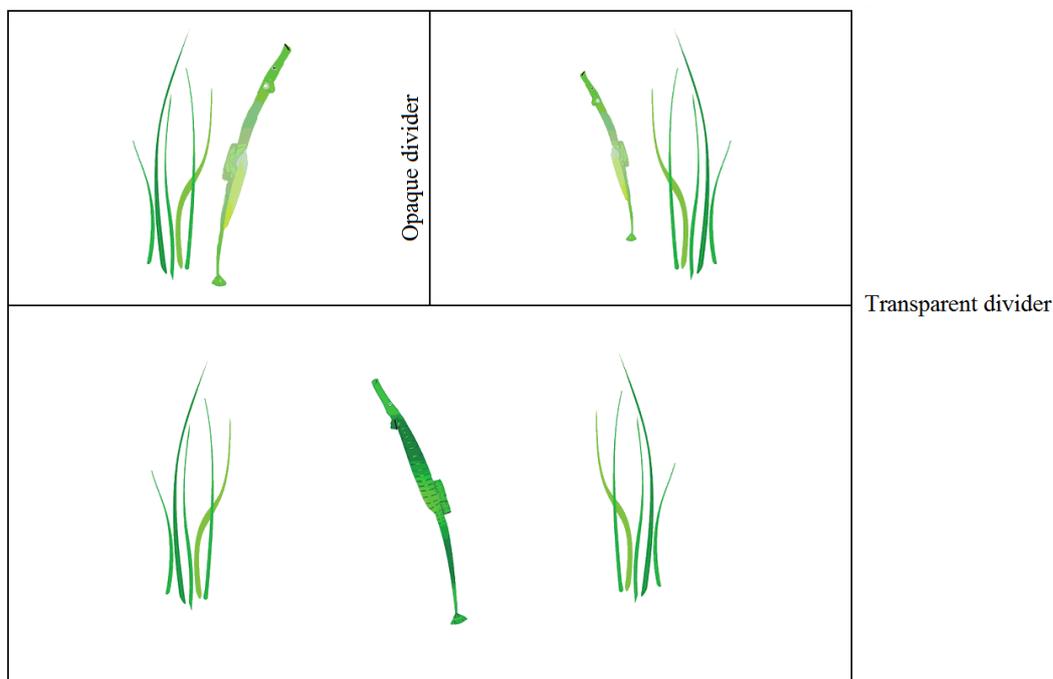
Figure 1. A focal fish (front) could choose between a large (rear left) and a small (rear right) partner of the opposite sex. Here the focal fish is a female choosing between two males.

The six aquaria – one for each treatment – were placed in a climate room where the temperature was set to 10-11˚C. Deep water (32 m) from the Baltic Sea was warmed using immersion heaters to approximately 17.5˚C and 25˚C, respectively. The deep water for the cold treatment was cooled down to approximately 10˚C by using bottles containing ice. The water for all treatments was stored in barrels, oxygenated by air-stones and pumped into the aquaria before a replicate was run. A particular aquarium always had the same temperature, but I randomly assigned to which aquaria the focal males and the focal females were placed. I also randomly assigned on which side of the aquarium the large and the small stimuli fish should be. Randomization was made using www.random.org. Before the onset of each replicate the light was turned on and the air, the dividers and the positions of the plastic eelgrass was checked and corrected.

Before each replicate was run, 2 l of water from the pipefish storage tanks was added to a plastic bag. One fish was put in each of three such plastic bags (one focal, one large, and one small stimuli fish) for each replicate. The bags were closed with clips and put in the right position in the experimental aquarium. The fish were kept in the plastic bags for 30 min to get acclimatized to the new temperature. After that they were carefully released into the aquarium. Another acclimatization period followed, this time 15 min. After that, observations began directly. Shower curtains were hanging in front of the aquaria with holes at eye-height, so I could observe the fish without disturbing them. I observed the position (left or right) and activity (rest, swim, court or dance) of the fish once every 10 minutes for 90 min (9 observations). After that, the fish were taken out of the aquaria but still kept at the temperature to which they were acclimatized. The fish were measured to the closest mm. Two kinds of infections were controlled for – the skin parasite *Cryptocotyle lingua* (visible as black spots in the skin) and an unknown disease clouding one, rarely two, eyes of the fish (here called the White eye). The males' pouches were also controlled to make sure no immature males were used in the experiment. Immature males have very flat pouches that are hard to see. Mature males have a conspicuous pouch, readily visible. As I tried to discriminate against immature males even before the experiment, no male had to be excluded afterwards because of this. After the measurements the fish were acclimatized back to the storage tank's temperature and then put back in them. All experimental fish were kept separately, and no stimuli-fish was re-used. Some focal fish were re-used as stimuli-fish. Also, 15% of the fish that I used had previously been used in another behavioural experiment (Sundin et al., submitted). After the experiments, the fish were released back into the wild.

The data were analyzed using R (R Development Core Team, 2008) and Statistica v.10.

Replicate sizes

I excluded replicates where the focal fish did not change position in the aquarium at least once. I also excluded experiments where any of the fish got sick, or where the replicate failed for other reasons. For the treatment with the female as focal, I ran 27 replicates in cold water and could use 22 them in the analysis. In intermediate tempered water I ran 27 replicates and could use 23, and for the warm treatment 26 of 27 replicates could be used. For the treatments with the male as focal, 21 out of 33 replicates in cold water, 22 out of 27 in the intermediate tempered water, and 22out of 27 in the warm water treatment could be used.

For the activity analysis I did not exclude any of the treatments where the fish did not change position, which left me with 27 replicates for all treatments with females as focal. For the male treatments I was left with 32 replicates in cold water, 26 replicates in intermediate-temperature water and 26 replicates in warm water.

For the infection based mate choice, I selected replicates from the temperature treatment replicates. The criterions were that one of the stimuli fish had to be visibly infected, while the other appeared to be healthy. I used 23 replicates where the males were the focals, and 31 replicates where the females were the focals.

Results

Temperatures

The temperature in the cold treatment was $10.3°C \pm 0.6°C$ SD (n= 120). In the intermediate tempered treatment it was $17.1°C \pm 0.8°C$ SD (n=108), and in the warm treatment $23.3°C \pm 1.6°C$ SD (n=108; Figure 2).
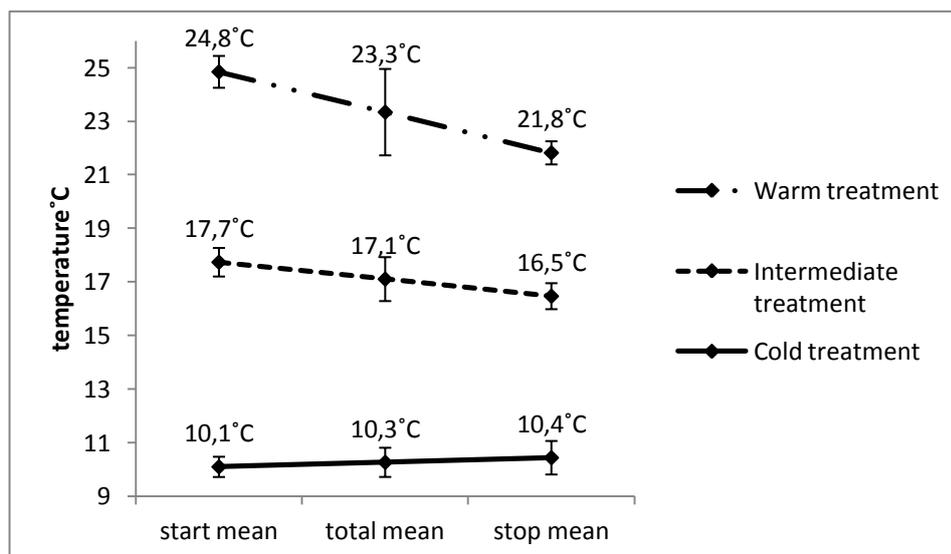

Figure 2. Mean start, total and stop temperatures in the cold, intermediate and warm treatments. The error bars shows the standard deviation.

Measurements

The females were 143 ± SD 15mm, and the males were 139 ± SD 13mm. Female and male length were significantly different (SS=2448, df=1, MS=2448, F=11.99, P<0.001, ANOVA), reflecting natural size differences. The length of the fish used did not differ between different temperature treatments (SS=196, df=2, MS=98, F=0.48, P=0.62, ANOVA). There was no combined effect of sex and temperature on the length (SS=24, df=2, MS=12, F=0.06, P=0.94, ANOVA).

The stimuli females were significantly longer than the stimuli males (SS=2242, df=1, MS=2242, F=23.7, P<0.0001, ANOVA), which is in accordance with natural lengths. The large stimuli fish were significantly larger than the small stimuli fish (SS=57702, df=1, MS=57702, F=611, P<0.0001, ANOVA).

The lengths of the stimuli fish did not differ between temperatures, sexes or within size categories (SS=150, df=2, MS=75, F=0.79, P=0.45, ANOVA; Figure 3).

Focal females were significantly longer than focal males (SS=2448, df=1, MS=2448, F=11.99, P<0.001). The length of the focal fish did not differ between different temperature treatments (SS=196, df=2, MS=98, F=0.48, P=0.62, ANOVA), and there was no interaction between sex and temperature (SS=24, df=2, MS=12, F=0.06, P=0.94, ANOVA).
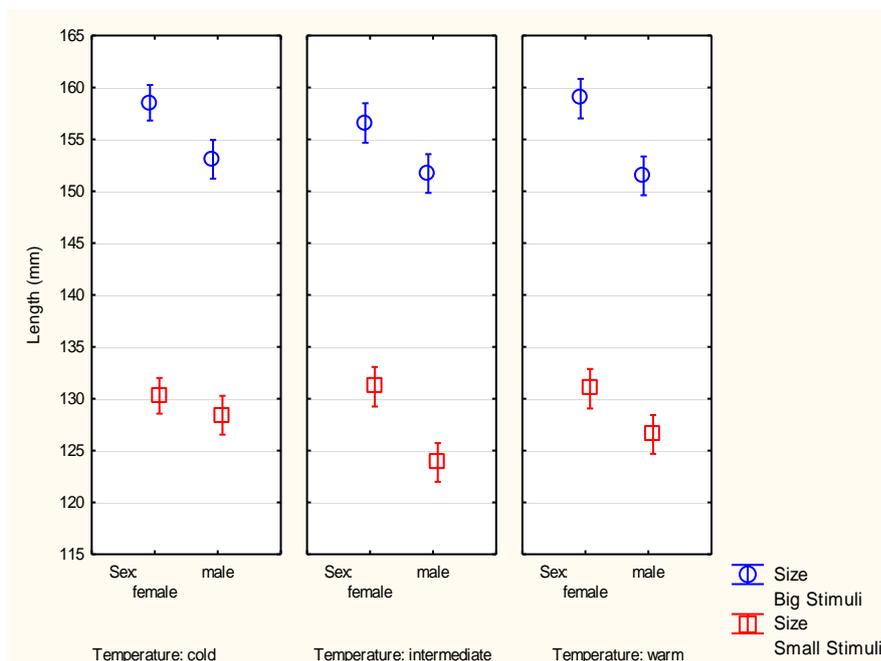

Figure 3. There is no combined effect of temperature, sex and size (e.g. large or small stimuli fish) on the length of the stimuli fish. The error bars show the standard error.

Infections

The males were significantly more infected with *Cryptocotyle* than the females (22.7% of the males and 13.9% of the females; W = 28293, P=0.012, Wilcoxon rank sum test with continuity correction; Figure 4). There was no significant difference in the number of females and males that carried the White eye (11.6% of the females and 11.3% of the males; W = 31094, P=0.9, Wilcoxon rank sum test with continuity correction). In total 23.5% of the females and 29.6% of the males were carrying either or both *Cryptocotyle* and the White eye (Figure 4). There was no significant difference in the number of fish that were infected in the cold, intermediate tempered and warm treatments (H=0.33, n=498, P=0.85, Kruskal-Wallis test).
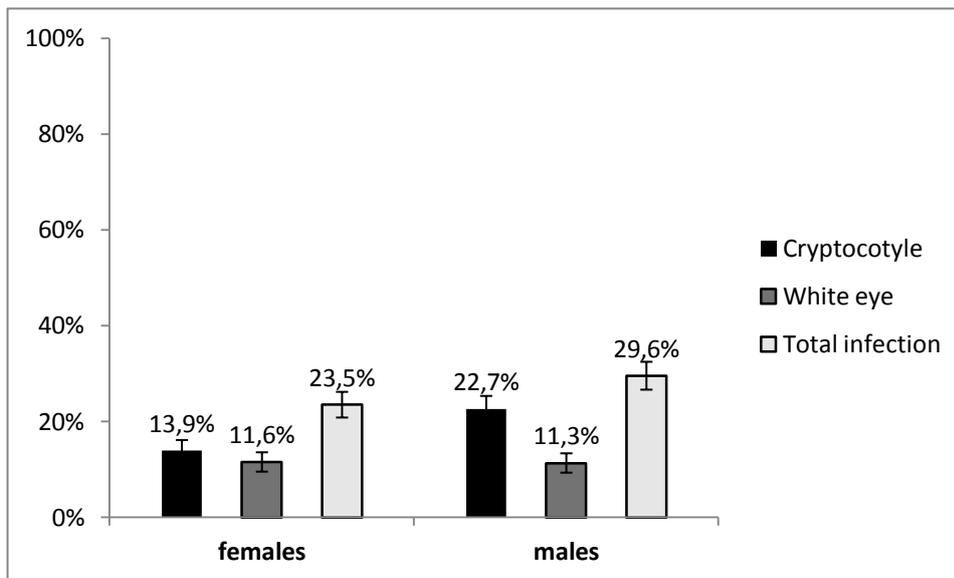
Figure 4. Proportion of females and males respectively that was carrying the parasite *Cryptocotyle lingua* or the White eye and proportion of females and males respectively that carried either or both of the two infections. The error bars shows the standard error.

Choice based on size

Neither male nor female pipefish exhibited a significant preference for the large stimuli partner (SS=0.73, df=1, MS=0.73, F=2.86, P=0.09, ANOVA; Figure 5). Moreover, temperature had no significant effect on choice (SS=0.03, df=2, MS=0.15, F=0.06, P=0.94), nor had any combined effect of temperature and sex on pipefish preference for the large stimuli fish (SS=0.43, df=2, MS=0.22, F=0.85, P=0.43; Figure 6). However, if I single out the females in the cold water treatment I find that they spent significantly more time with the large male (W = 348, P=0.013, n=22, Wilcoxon rank sum test with continuity correction).
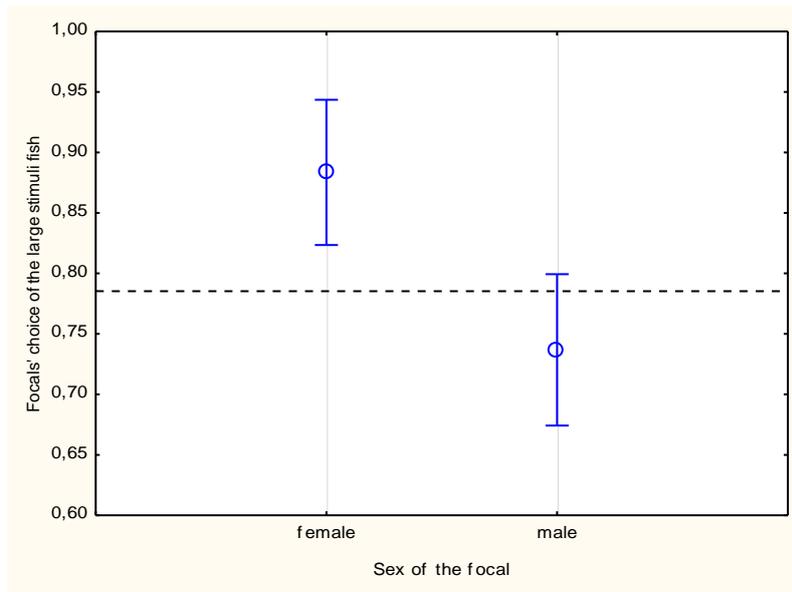
Figure 5. Arcsine square-root transformed proportions of how many times the focal females and males were observed in front of the larger stimuli partner. The error bars show the standard error. The horizontal line represents a random choice.
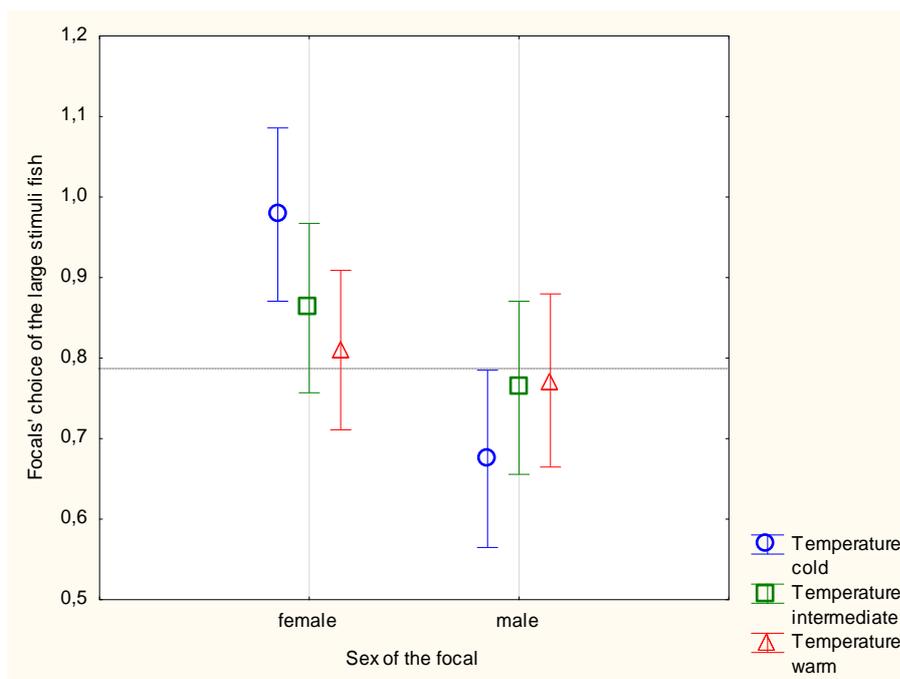

Figure 6. Arcsine square-root transformed proportions of how many times the focal females and males were observed in front of the larger stimuli partner in each temperature treatment. The error bars show the standard error. The horizontal line represents a random choice.

Choice based on infection

I tested whether the presence of *Cryptocotyle* and the White Eye on the stimuli fish had any effect on the choice of the focal fish. There was no significant effect of sex on the choice of the un-infected (*Cryptocotyle* + White eye) stimuli fish (SS=0.63, df=1, MS=0.63, F=2.09, P=0.15, ANOVA), i.e., neither males nor females showed any significant preferences for un-parasitized partners. However, if I single out the focal males, I find that there was a non-significant tendency for them to be observed more often in front of the un-infected stimuli

female (W = 348, P=0.065, n=23, Wilcoxon rank sum test with continuity correction; Figure 7). The infected females were 148mm ± 16mm, which was significantly longer than the length of un-infected females (142mm ± 15mm; t = 2.6, df = 249, P=0.01 Figure 8). The infected males were 143mm ± 13mm, which was significantly longer than the length of un-infected males (137.5mm ± 13mm; t = 2.8, df = 245, P<0.01, Two Sample t-test; Figure 8).
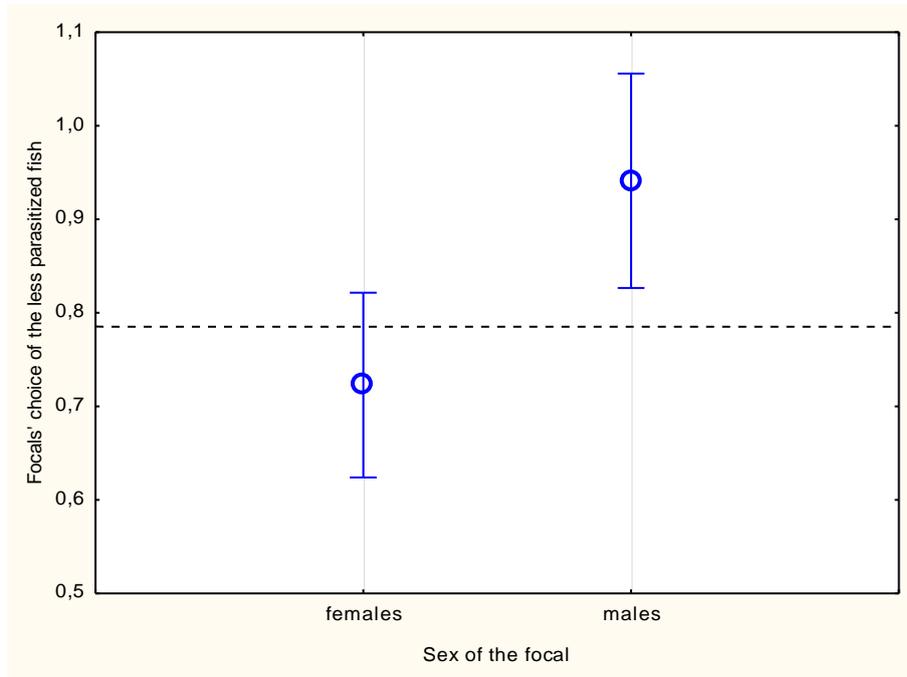

Figure 7. Arcsine square-root transformed proportions of how many times the focal females and males were observed in front of the un-infected stimuli fish. The error bars show the standard error. The horizontal line represents a random choice.
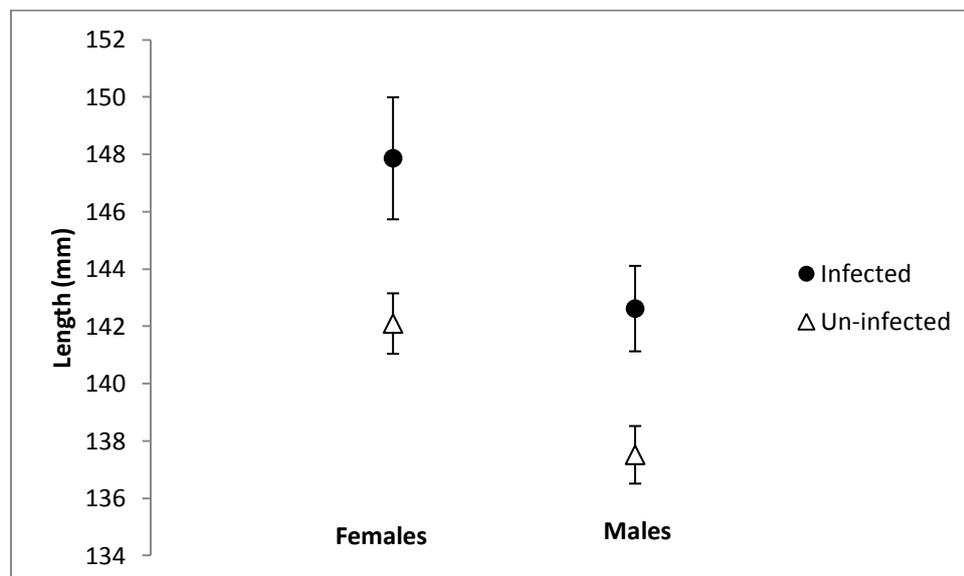

Figure 8. The length of the infected females and males (i.e. carrying *Cryptocotyle* and/or the White Eye), and the length of the un-infected females and males. The error bars show the standard error.

Activity

Sex had no effect on activity (SS=0.24, df=1, MS=0.24, F=0.96, P=0.32, ANOVA). Temperature, however, had: pipefish were significantly more active in warmer water (SS=1.76, df=2, MS=0.88, F=3.6, P=0.03, ANOVA; Figure 9). There was no combined effect of sex and temperature on activity in pipefish (SS=0.24, df=2, MS=0.12, F=0.49, P=0.61, ANOVA). Activity is here defined as any kind of active movement made by the fish.

Sex had no effect on the amount of courtship (SS=0.001, df=1, MS=0.001, F=0.02, P=0.9, ANOVA). However, there was a non-significant tendency towards an effect of temperature on the amount of courtship (SS=0.28, df=2, MS=0.14, F=2.64, P=0.074, ANOVA; Figure 10), but there was no combined effect of sex and temperature on amount of courtship (SS=0.04, df=2, MS=0.02, F=0.4, P=0.67, ANOVA).
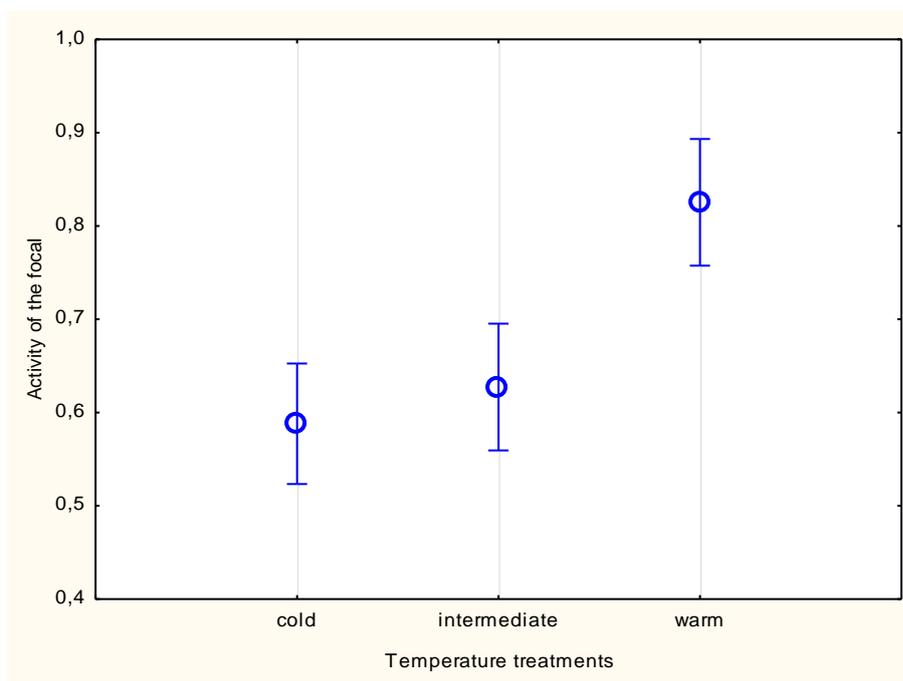

Figure 9. Arcsine square-root transformed proportions of the number of times the focal fish was observed to be active. The error bars show the standard error.
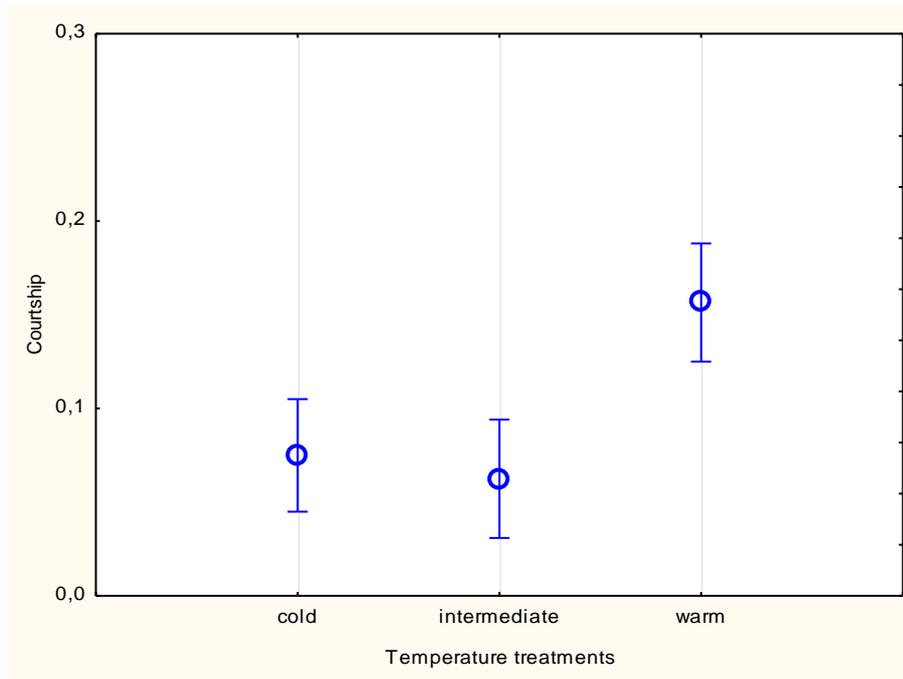
Figure 10. Arcsine square-root transformed proportions of the number of times the focal fish was observed to be courting. The error bars show the standard error.

Discussion

I have investigated whether sex and temperature affected mate choice (e.g. large vs. small mate) and activity (e.g. any kind of active movement made by the fish) in a Baltic Sea population of the broad-nosed pipefish *Syngnathus typhle.* I found that neither males nor females preferred a larger partner, and that sexes did not differ in activity. Temperature had a clear effect on activity: both males and females were more active in warmer water. There was no significant effect of temperature on mate choice, but females in cold water tended to prefer larger males.

Parasites had no effect on mate choice in this species, but when the sexes were analysed separately males tended to spend more time in front of the less parasitized females.

These results are surprising: firstly, neither males nor females seemed to be choosy in general (e.g. seemed not to care about the size of the mate), and secondly temperature did not seem to have any effect on choosiness. In fact, the observed tendency, i.e., that females in cold water preferred large males, was in the opposite direction from the predicted. These findings contrast sharply to what has been found in the west coast population of *S. typhle*: there, a clear preference for larger mates exists (Berglund et al. 1986), and especially males discriminate between large and small females (Berglund and Rosenqvist 1993). This preference seems to be mostly absent in the Baltic population according to this study. Moreover, in the west coast population female PRR exceeded male PRR more in cold than in warm water (Ahnesjö 1995), so that males would be predicted to be choosier in cold water than in warm water, again a contrast to this study where, if anything, females in cold water were choosy whereas males were not.

Why do I find these unexpected patterns in the Baltic population of *S. typhle*? This is presently unclear, but perhaps the stressful Baltic brackish environment plays a role. Few organisms are fully adapted to the low salinity in the Baltic, because the Baltic Sea has only existed in its current state for less than 10,000 years (Migula et al. 2003, Bernes 2005), probably a time too short for successful evolutionary changes. Organisms are either adapted to marine conditions or to fresh water conditions. Consequently, the Baltic *S. typhle* are smaller than their west coast counterparts, a pattern found in several species (Bernes 2005). It is possible that yet unknown traits, other than size, are playing a role in mate choice in the Baltic, or simply that choosiness in this population for some reason is low or absent. Males of the Baltic population have been shown to not be able to tell the difference between males and females by using smell (Lindqvist et al. 2011), although they could by using vision. Whether the west coast population of *S. typhle* can tell the difference between the sexes by using smell is not known.

The next question to ask is why the Baltic *S. typhle* did not discriminate significantly between parasitized and non-parasitized partners, as did the males on the west coast (Rosenqvist and Johansson 1995). Maybe, with a larger dataset, the males would be found significantly more on the less parasitized females' side of the aquaria, in accordance with Rosenqvist and Johansson (1995), or maybe they are just not as choosy with regards to parasites in the Baltic population. The west coast population exhibited a negative correlation between female fecundity and parasite load (Rosenqvist and Johansson 1995), a possibility also in the Baltic Sea population of *S. typhle*. Why females do not discriminate between parasitized and non-parasitized males needs to be further investigated.

The activity (e.g. any kind of active movement made by the fish) did not differ between males and females, but both sexes were more active in warmer than in colder water. This is probably due to lower metabolic rates in cold water (Lewis and Driedzic 2007) and represents the typical pattern in poikilotherms. Consequently, the amount of courtship in both sexes was higher in warmer water, as courtship is a form of active movement. However, I expected females to court more than males, as this species is considered as sex role reversed. Surprisingly, males and females courted each other equally much, hinting that the sex roles might be more equal in the Baltic population. To investigate this, and to measure male and female PRR and to estimate the strength of sexual selection in Baltic pipefish forms interesting tasks for future research.

Conclusions

I have found that activity and courtship did not differ between sexes, but that the fish were significantly more active, and thus also courted more, in warm water.

I also found that sex, temperature and parasite load did not affect mate choice in the Baltic Sea population of the pipefish *Syngnathus typhle*. However, females in cold water preferred larger males, and there was a trend for males to prefer less parasitized females. My main results contrast to earlier findings in a west coast population of this species. Why this is so remains unclear, but suggests that the pattern of sexual selection is different in the Baltic

population. Much more research is needed to understand how mate choice works in this population, and in what ways and to what extents they differ from the population in the Swedish west coast.

Acknowledgements

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