

Sexual signalling and noise pollution in the sea

- Implications for courtship behaviour and reproductive success in two vocal species of gobies

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**Department of Biological and Environmental Sciences
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DISSERTATION ABSTRACT

Many marine animals use acoustic signals to mediate social interactions. Acoustic cues and signals are especially important in water because sound is unique as a sensory modality propagating with little attenuation over long distances, at all depths, and irrespective of the water current direction. Anthropogenic underwater noise is a global pollutant of increasing concern but its impact on reproduction in fish is largely unknown. Hence, a better understanding of this important link to fitness is crucial. Here, I compared different courtship traits, including courtship sounds, in two sympatric *Pomatoschistus* species and I found that courting males of the common goby *Pomatoschistus microps* sing louder and produce sounds of shorter duration than males of the sand goby *Pomatoschistus minutus*. Furthermore, eyes of *P. minutus* females turn black during courtship attempts, whereas this is not the case for females of *P. microps*. Dark eyes in females of *P. minutus* were more likely to be displayed by more gravid females, but males did not respond behaviourally or preferred dark-eyed females. I suggest that dark eyes are not a signal per se but may be an aspect of female mate choice, possibly related to vision. Furthermore, I examined if an experimentally altered body condition in *P. minutus* males affects acoustic and visual display and if it influences females' decision to spawn or not. Visual and acoustic courtship and reproductive success was studied under two experimental food regimes (high food and low food) and compared to a control group (fish from the field). Condition did not affect visual or acoustic courtship, nor did it affect mating success. Females only spawned with males that produced sound and courtship sounds are likely to be important in female mate choice. To further understand how anthropogenic noise can affect mating success by masking the acoustic cue, I experimentally tested the impact of broadband noise exposure on the behaviour and reproductive success of *P. microps*. Noise treatment had similar frequency range as anthropogenic boat noise and was presented either continuously or intermittently. The continuous noise treatment had the most detrimental effect by reducing spawning probability, whereas male nest-building behaviour and active pre-spawning behaviour (including courtship) were unaffected. Additionally, females took longer to spawn under continuous noise than in the control. Egg density was significantly higher in both noise treatments compared to the control. Since sexual selection can be sensitive to changes in the environment I also investigated effects of noise on male mating success in *P. minutus*. I compared no added noise ('silence') to added artificial Brownian noise to create disturbance at moderate levels. In silent condition, successful males were significantly larger than unsuccessful males, which was not the case in the noise treatment. More males received eggs in the silent treatment compared to the noise treatment, creating a relaxed opportunity for sexual selection in the silent environment. However, here was no significant effect of treatment on the number of spawned eggs. The results suggest that disturbance caused by noise can influence mating decisions and traits under sexual selection. In conclusion, in this thesis I show that noise, particularly a continuous noise exposure, negatively affects reproductive success, highlighting its potential to impact fish demography. Future studies in natural conditions are required for a better understanding of the impact of noise on fish reproduction. Thus, I suggest that aquaria studies should be performed in a low noise environments, since noise clearly can affect the outcome of an experimental result.

LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- Paper I: E-L. Blom, I. Mück, K. Heubel and O. Svensson**
Courtship sound and associated behaviours of two sympatric marine Gobiidae species – *Pomatoschistus microps* and *Pomatoschistus minutus*.
Environmental Biology of Fish (2016) 99: 999–1007.
- Paper II: K. H Olsson, S. Johansson, E-L. Blom, K. Lindström, O. Svensson, H. Nilsson Sköld and C. Kvarnemo**
Dark eyes in female sand gobies indicate readiness to spawn. *PLoS One*, (2017) 12: e0177714.
- Paper III: E-L. Blom, J. Wilson, K. de Jong, C. Kvarnemo, T. Johansson, M.C.P. Amorim and O. Svensson.**
Acoustic display is an important cue for mate choice for females of *Pomatoschistus minutus*, but acoustic display is not affected by male condition. *Manuscript*
- Paper IV: E-L. Blom, C. Kvarnemo, S. Schöld, M. H. Andersson, O. Svensson and M. C. P. Amorim**
Continuous and intermittent noise has a negative impact on reproductive success in a marine fish with paternal care.
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- Paper V: K. de Jong, E-L. Blom, M. Nygård, M. H. Andersson, C. Kvarnemo and O. Svensson**
Disturbance caused by noise affects sexual selection in a marine fish. *Manuscript*

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INTRODUCTION

In this thesis, I investigate the spawning behaviour and the associated acoustic courtship of the common goby, *Pomatoschistus microps*, (Krøyer 1838), and the sand goby, *Pomatoschistus minutus* (Pallas 1770). I also want to understand how spawning behaviour and the acoustic courtship can be affected by anthropogenic noise, with application to wild populations. But also, considering the effects in noisy experimental situations.

Animals use a wide range of modalities during courtship and the effectiveness of signals co-varies with environmental conditions. Therefore, signals (including acoustic signals) often match measures of optimal signal transmission (Bradbury & Vehrencamp 2011; Wilkins et al. 2013). For example, under turbid conditions, visual courtship signals can be hampered (Seehausen et al. 1997; Järvenpää & Lindström 2004; Heubel & Schlupp 2006) and changes in pH-levels can modify the use of chemical cues (Heuschele & Candolin 2007).

Sexual selection and Sexual signalling

Darwin (1871) defined sexual selection as “the advantage which certain individuals have over other individuals of the same sex and species solely in respect of reproduction”. When a trait is favoured in a reproductive context it is subject to sexual selection. Such 'secondary sexual traits' are often most strongly selected in males and can be driven beyond what is optimal for the animal to survive, such as colourful ornaments that likely attracts predators as well as mates. Darwin (1871) identified two different mechanisms of sexual selection, mate choice (inter sexual selection) and contest competition (intra-sexual selection). After mainly focusing on male competition, the impact of female mate choice on reproductive evolution has grown, as female choice drives male traits in future generations (Zahavi 1975; O'Donald 1980; Kirkpatrick 1982; Pomiankowski 1987; Grafen 1990; M Andersson 1994).

Cues and signals

Displays in a sexual context are often highly complex and involves many components, cues and signals. A signal is often based on multimodal components but is evaluated as one signal. A signal can evolve if it alters the behaviour of another organism and if the receiver of the signal has evolved a response (Smith & Harper 2003).

In many species, both marine and terrestrial, as a part of mate selection, males often perform a conspicuous courtship display including bright ornaments and a courtship song for females. Males can also offer resources such as a territory or a nest, which may be used as a cue for male condition (Jennions & Petrie 1997; Moller & Pomiankowski 1993; Kodric-Brown & Nicoletto 2001). Both signals and cues provide information to potential mates. A cue can be a by-product of for example foraging that a predator can perceive and use for own benefit. A signal contains information about a specific purpose and therefore also has an influence on the receiver's behaviour which in turn has an impact of fitness of the species relative to both the receiver and the signaller (Laidre & Johnstone 2013). A signal may however evolve from a cue. Some traits, such as colourful ornaments or courtship behaviour, are not fundamental for the individual to survive but have been favoured because the trait increases opportunities to be selected under mate choice. Some traits that are under survival selection such as speed or size, might also be evaluated as fitness and therefore also selected even though it has not arisen as a signal (Candolin 2003).

There are three main mechanisms that have been proposed to explain mating preference and the selection of sexual signals. These mechanisms may work alone or in combination.

1. Direct benefits - 'the good parent'. This mechanism is mainly based on parental care where the male invests in his offspring by rearing the fertilized eggs which enhances offspring survival (Hoelzer 1989). Thus the fitness of the female will be higher if she is able to select the high-quality male (Kirkpatrick 1987).
2. Indirect or genetic benefits - 'the good gene'. A female can improve her offspring's fitness by choosing a male with the 'the best genes' if the male's signal carries information about his genetic quality (Zahavi

1975) and therefore benefit from more attractive offspring (Fisher 1999) [the runaway process of Fisher (1930)];

3. Sensory drive - sexual selection will favour males that express signals that females have an *a priori* preference for and which match these sensory biases (Ryan & Rand 1993).

Visual displays

Females assess different attributes during male courtship. The male has to signal his condition as an information that the female can understand. This signal must be an honest representation if it is to function as a reliable predictor of quality, and the female has to be able to discriminate between honest and dishonest signals (Smith and Harper 2003). For example, Andersson (1982) showed that visual colour displays in birds are sexually selected signals. Knapp and Kovach (1991) showed that male courtship rate is an honest display in male damselfish (*Stegastes partius*) and that females choose males with a high courting rate rather than males with a low courting rate. Males with a high courting rate also had a significantly higher egg survival and therefore were shown to be better parents. This in turn showed that a higher display is costlier to produce and reflects on parental ability. Visual colour display impact on fitness has also been demonstrated in guppies, where males with a higher content of carotenoid pigments are found to have a stronger immune system and produce more viable sperm (Hudon et al. 2003; Grether et al. 2004; Locatello et al. 2006). This signal is expressed in their body colour as orange spots, and female guppies have been shown to prefer males with more orange coloration (Grether et al. 1999).

Vocal displays

Sexual displays often involve many components and are highly complex signals (Candolin 2003). For example, in bird species males are often both brightly ornamented and perform an elaborate song, whereas many fish species combine bright colours with conspicuous courtship displays (Oliver & Lobel 2013). In addition, males of several species offer some resource to the female, such as a territory or a nest, which may be used as a cue in female mate choice. Females might evaluate signals differently depending if the

choice is directly beneficial ('good parent') or indirect ('good gene'). When birds perform their song repertoire it is a cue for the female about both the genetic quality of the male but also about the territory characteristics (Searcy 1992, Candolin 2003).

Acoustic cues and signals are especially important in water. Sound in water is unique as a sensory modality in propagating with little attenuation over long distances, at all depths, and irrespective of water current direction (Rogers & Cox 1988). Acoustic communication is used by many species of fish (Bass 2008 et al., Amorim et al. 2015). The full functions of these sounds are yet not fully understood but it has been proposed that they are used in female mate choice and may also possibly be used for species recognition, given the clear inter-specific differences in breeding sounds (Lugli & Torricelli 1999; Lindström & Lugli 2000; Pedroso et al. 2013).

Vocal signal interference

An additional and growing component in the marine soundscape is anthropogenic noise derived from human activities, such as shipping and recreational boats, as well as sources such as pile driving and seismic airgun (Popper & Hastings 2009; Radford et al. 2014). Regardless of the source, anthropogenic noise creates temporary and unpredictable fluctuations in the acoustic environment, leaving almost no marine area unaffected (McDonald et al. 2006). Depending on the source and its characteristics, anthropogenic sound can have varying impact on the environment in which the sound is emitted. Besides mate choice and species recognition, acoustic signals are known to be used by fish in rival assessment, foraging and navigation (Popper & Hastings 2009). Many anthropogenic sound sources overlap with the frequencies fish produce and are able to detect (Slabbekoorn et al. 2010) (figure 1) resulting in lost cues or signal detection. Lost cues can lead to missed mating opportunities having a direct effect on fitness. This overlap also force species using auditory signals to compensate for the increased background noise, including changes in the signal frequency, signal modality and temporal adjustments to signal production (Radford et al. 2014). Anthropogenic noise can also disrupt other auditory cues which marine organisms rely on to survive. Fish larva settlement for example is induced by reef sounds (Simpson et al. 2005) and this cue can be masked by anthropogenic noise.

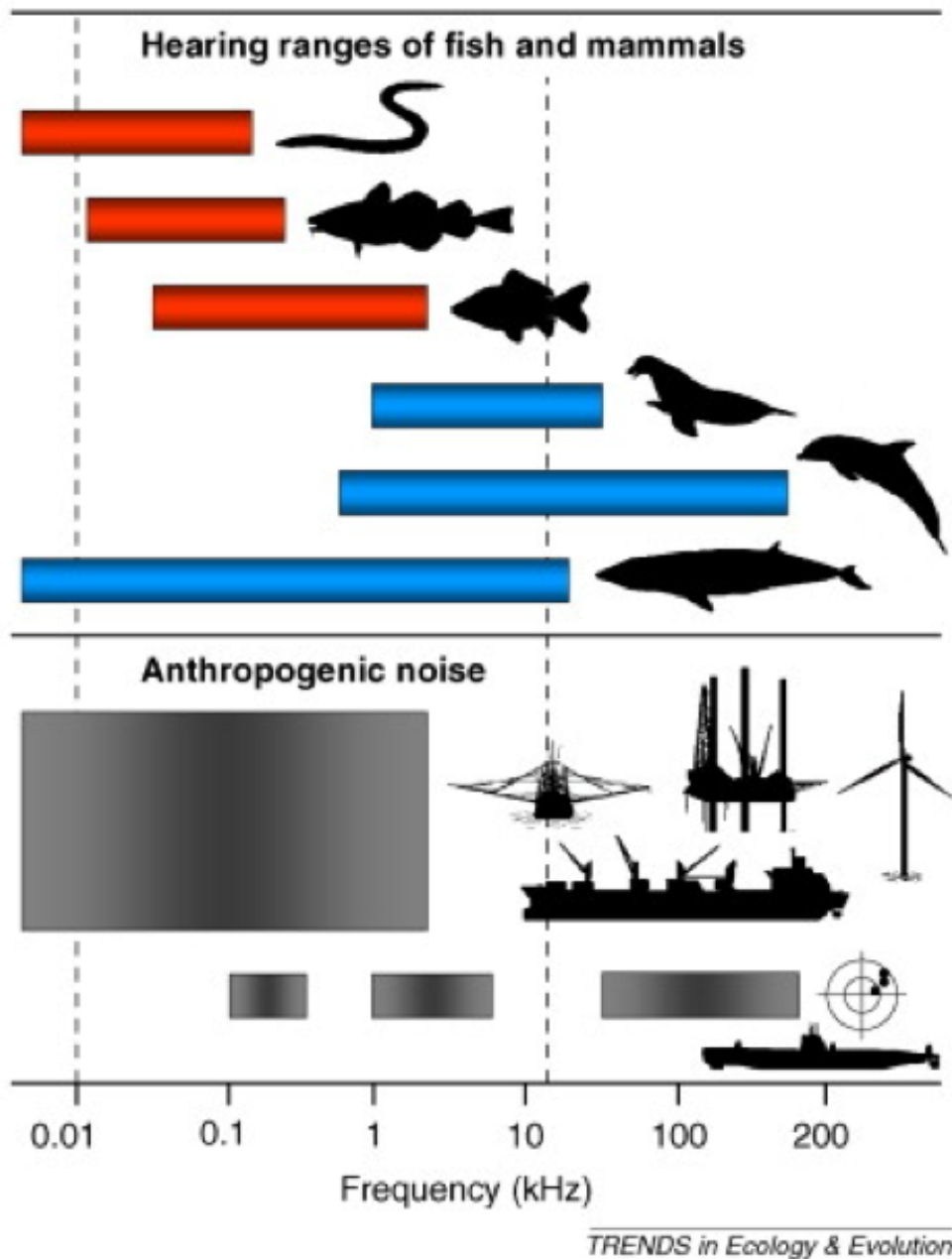


Figure 1. Hearing ranges of selected fish and mammal species. Vertical dashed lines demarcate the human hearing range in air. Each species has a more restricted range of peak sensitivity within the species-specific limits (not indicated). From top-to-bottom, red horizontal bars represent: European eel, a freshwater species spawning at sea with sensitivity to infrasound; Atlantic cod, a marine species with ‘average’ hearing abilities; and goldfish, representing many freshwater fishes with specially evolved hearing abilities. For mammals in blue; Californian sea lion, bottlenose dolphin and fin whale. The anthropogenic noise ranges indicate where the majority of sound sources have most of their energy, although some human generated sounds exceed these frequencies. At the bottom of the figure are frequency ranges of low-frequency (USA), mid-frequency and high-frequency sonar. This figure is reprinted with kind permission from Hans Slabbekoorn, taken from ‘A noisy spring: the impact of globally rising underwater sound levels on fish’ (Slabbekoorn et al. 2010).

Sound in the sea

The ocean is filled with different sounds originating from both abiotic and biotic sources. Abiotic sound sources are mainly from surface motion with breaking waves, rain, thunder storms and wind, while biotic sounds arise from marine mammals, fish and invertebrates. All these sounds are natural parts of the marine acoustic environment (Hildebrand 2009a; Ladich 2013) and are referred to as ambient noise. Organisms living in the ocean use this acoustic landscape to navigate, find suitable habitats and food, and avoid predators (Simpson et al. 2005; Slabbekoorn et al. 2010). In addition, as mentioned previously, many marine animals use acoustic signals to mediate social interactions, such as mate finding and mate choice, territory defence and predator warning (Bass & McKibben 2003; Ladich 2013; Amorim et al. 2015).

Anthropogenic sound sources, their characteristics and effects

Low-frequency sound (10-500 Hz) can propagate very long distances and has a long-range effect. Because its absorption is weak, low-frequency sound often creates more chronic noise compared to mid (500-25 kHz) and high (>25 kHz) frequency sounds, which tend to have a more local effect (Hildebrand 2009b; Haviland-Howell et al. 2007). Sources of anthropogenic noise at lower frequencies derive mainly from shipping but also from seismic airguns and vibratory pile driving (Hildebrand 2009a).

Sources of mid-frequency anthropogenic noise are sonars, leisure crafts (small boats and jet-skis used for fishing and recreational activities) and acoustic harassment devices (AHDs) (Hildebrand 2009a). These sources act as chronic noise sources mainly during high traffic and even though these mid-frequency sounds do not propagate far they still contribute to a high local impact (Haviland-Howell et al. 2007).

Seismic airguns create not only low-frequency but also high frequency anthropogenic noise. Seismic airguns have a widespread usage, especially in the search for oil at different layers of the seafloor. In recent years, the use of seismic airguns has moved into even deeper waters where it has a great potential to be propagated further. The highest source levels for high frequency sounds derive from pile driving and more rarely from use of

explosives. Construction pile-driving is mainly used in shallow water, limiting its effect as primarily local (Hildebrand 2009a).

Biological responses to anthropogenic noise

Mammals, fish and invertebrates exposed to anthropogenic noise in aquatic and terrestrial environments can show both physiological and behavioural changes (Williams et al. 2015; Radford et al. 2016). The response depends mainly on how noise is perceived and can be anything from physiological damage to alteration of important behaviours, such as foraging and movement, or inability to detect cues from the surroundings. Noise can function as a direct stressor, causing elevated stress hormone levels or pain (Smith et al. 2004; Wale et al. 2013). Some animals perceive noise as a threat, causing escape behaviour (Berthe & Lecchini 2016). Possible outcomes from low intensity exposure is hearing loss, stress and immune system changes (Popper & Hastings 2009). Exposure to high intensity sounds have been shown to cause acute changes in movement patterns such as schooling and jetting, which then cease when the noise ends (Fewtrell & McCauley 2012). More concerning are sustained reactions, such as body malformation (de Soto et al. 2013), which in turn might have a long-term effect on the individuals' health. Outcomes from high intensity sound exposure may include temporary hearing loss, tissue damage or even death (Popper & Hastings 2009). Since boat traffic is increasing and oil and energy exploration is moving into deeper water, long-term chronic exposure is becoming a reality for most marine organisms (Haviland-Howell et al. 2007; Hildebrand 2009a).

Chronic exposure may also result in habituation. Signs of habituation have been found in the shore crab (*Carcinus maenas*), which had as an initial response to ship noise increased its oxygen consumption – a sign of physiological stress – whereas continued exposure did not maintain the stress response (Wale et al. 2013). Similarly, the common cuttlefish (*Sepia officinalis*) showed an escape response when first exposed to noise, but this response declined when the noise was played again (Samson et al. 2014). However, if the exposure is continuous, there might be other responses that occur instead, indicating that full tolerance to noise is not a likely outcome (Anderson et al. 2011).

Continuous noise vs. intermittent noise

Some types of anthropogenic noise are continuous, whereas other types primarily occur intermittently. Research studies on whether continuous and intermittent noise affect marine organisms differently are limited and the results are contradictory. It has been shown that intermittent noise has a larger effect on the cortisol levels in the giant kelp fish (*Heterostichus rostratus*) than continuous noise (Nichols et al. 2015), while the red drum (*Sciaenops ocellatus*) showed no difference in cortisol levels in response to the same treatments (Spiga et al. 2012). Furthermore, behavioural studies on invertebrates have shown that both continuous and intermittent noise affect behaviours, but again, that the specific response varies markedly between species, despite being tested in the same setting (Solan et al. 2016). The contradictory results are important as they highlight that interspecific differences should be expected, given that some species are likely to be more sensitive to intermittent or continuous noise than others.

AIMS

The overall aim of this thesis is to increase the knowledge of visual and acoustic courtship behaviour in both male and female fish. As sexual signals are an essential part of reproductive success and therefore fitness. I want specifically investigate how anthropogenic noise can affect sexual signals and how fish in experimental conditions can be affected by anthropogenic noise. To address the thesis, aim, the specific goals are as follows:

1. Describe and compare the sexual signals, including acoustic and visual courtship traits and behaviour of both males and females in *P. minutus* and *P. microps*, two highly sympatric goby species commonly used in experimental setups (**paper I**).
2. Given that *P. minutus* females, but not *P. microps* females, get conspicuously dark eyes that are displayed temporarily during courtship (**paper I**), experimentally test if male *P. minutus* prefer to associate with dark-eyed females, investigate if dark eyes are displayed during female aggression, and if dark eyes are associate with female readiness to spawn (**paper II**).
3. Test experimentally if manipulated body condition affects male acoustic courtship in *P. minutus* and investigate if the acoustic signal correlates positively with mating success (**paper III**).
4. Determine if exposure to continuous and intermittent broadband noise affects fitness related traits and reproductive success in the *P. microps* (**paper IV**).
5. Test experimentally if sexual selection is affected by noise in *P. microps* (**paper IV**) and *P. minutus* (**paper V**).

METHODS

The following section provides a general description of the empirical conditions of the study, including description of the study site, and housing and treatment of fish used in the experiments.

Study species

The sand goby (*Pomatoschistus minutus*) and the common goby (*Pomatoschistus microps*) are small marine fishes distributed in lagoons, coastal areas and estuaries of the Atlantic, Mediterranean and Baltic region (Miller 1986; Kullander et al. 2012). The two species are sympatric in the study area on the west coast of Sweden, with extensive overlap between their species' habitats. However, *P. microps* is more abundant in very shallow and often muddy areas, whereas *P. minutus* is more commonly found on sandy bottoms and in slightly deeper (>0.5 m) water (Miller 1986; Nellbring 1993). During a single breeding season, these short-lived fishes (1-2 years) can reproduce repeatedly with different mates (Miller 1975; Forsgren 1999). There is an overlap in the breeding season between the species, with spawning peaks occurring in spring and early summer (earlier peak in *P. minutus*, range March to July, and later peak in *P. microps*, May to September (Kullander et al. 2012).

Sound production and associated behaviour in species of the family Gobiidae is well described in six species of the sand goby group which have been shown to produce low frequency acoustic pulses in a reproductive context: marbled goby *Pomatoschistus marmoratus* (Risso 1810), Canestrini's goby *Pomatoschistus canestrini* (Ninni 1883), sand goby *P. minutus*, common goby *P. microps*, Adriatic dwarf goby *Knipowitschia panizzae* (Verga 1841), and Italian spring goby *Knipowitschia punctatissima* (Canestrini 1864) (Malavasi et al. 2012; Bolgan et al. 2013; Pedroso et al. 2013).

Study site and husbandry

All experiments were conducted at Sven Lovén Centre for Marine Infrastructure Kristineberg on the west coast of Sweden (58°15' N, 11°27' E) between May and July 2013-2015. All fish were caught by hand trawling at a depth between 0.2 m and 1.2 m in bays nearby the station. All fish

separated according to sex and housed in 50 l storage tanks prior to use in experiments. To guarantee natural light conditions, most experiments were conducted in a greenhouse. All aquaria had a continuous flow of natural seawater (salinity 22 – 31 ppt), and water temperature was measured daily. All fish (except those used in the experiment of **paper III**) were fed every second day with commercial fish food granules (Nutra HP, Skretting) and/or frozen *Artemia* sp.

Experimental setups

In **paper I, II, III** and **IV** all experimental aquaria (20 l) were separated by opaque screens to avoid visual interaction between fish in adjoining replicates and the aquaria were insulated from ground borne vibrations. Each experimental aquarium was equipped with a nest site made of a halved clay flowerpot or a polypropylene tube (Ø 56 mm). The polypropylene tube was fitted with a pipe attached like a chimney (Ø 20 mm) to fit the hydrophone. This design allowed for recording of courtship sounds that males made inside the nest. All nests also included a plastic sheet which lined the ceiling, for females to lay eggs on, making it easier to photograph clutches. In **paper V** transparent enclosures (plastic boxes, 78x56 cm bottom area, height 44 cm, with lids on) were used. The first experiment of **paper V** was done with two sets of four boxes placed 50 m apart in a shallow bay, whereas in the second experiment of **paper V**, the same boxes were placed in sets of four, inside small pools (1.5 m in diameter, water depth 20 cm) on land and provided with a continuous inflow of seawater. Each box also contained a 2-3 cm layer of sand, and four half flower pots as nest substrates (figure 3).

Treatments

For all experiments, all females and the males assigned to control treatment, were housed in storage tanks (50 l) for 7 days before experiment started, and fed daily. In **paper III**, control males were only kept in storage tanks for two days after capture and fed once, after which they were used in trials

Food regimes

Males were housed in storage tanks (50 l) 14 days before experiment started and were randomly assigned to one of two experimental holding tanks. In tank 1 all fish were fed every day, referred to as high food regime, in tank 2 they were only fed once a week, referred to as low food regime.

Noise

In **paper IV** and **V** noise were added as a disturbance. In **paper IV** I used a polypropylene ($\text{\O} 56 \text{ mm}$) tube, with a closed bottom end, filled with 1 dl of soft airgun balls was used. The tube was placed vertically in the right rear corner of the aquarium and noise (figure 2) was created by tumbling the soft airgun balls, by bubbling compressed air at the bottom of the tube. In **paper V** I placed a portable stereo player (Excibel KW 68-MP3U) and a portable speaker system consisting of two speakers and an amplifier (Philips, $>10\text{Hz}$) playing brown noise. The disturbance that was created by this set-up is likely a combination of noise and vibrations from the stereo transplanted through the plastic boxes.

Sound recordings and Sound analyses

All sound recordings were registered using a calibrated hydrophone (HTI-96-MIN with pre-amplifier, High Tech Inc., Gulfport MS; sensitivity $(-165 \text{ dB re } 1 \text{ V}/\mu\text{Pa})$, frequency range $0.02\text{--}30 \text{ kHz}$) connected to a digital audio recorder (Song Meter SM2+, Wildlife Acoustic, Inc., Maynard, US, sampling frequency 24 kHz). Note that the resonant frequency of the experimental glass aquarium was 4.9 kHz (paper IV) and 2.3 kHz in the plastic boxes (paper V). Sound analyses were performed using the Aquatic acoustic metrics interface (AAMI) software to calculate sound pressure levels (SPL) - SPL_{rms} (dB re $1 \mu\text{Pa}$) representing the average sound level for each fish. For frequency analysis, Matlab_R2016a (The Mathworks Inc., Natick, Massachusetts, USA) was used.

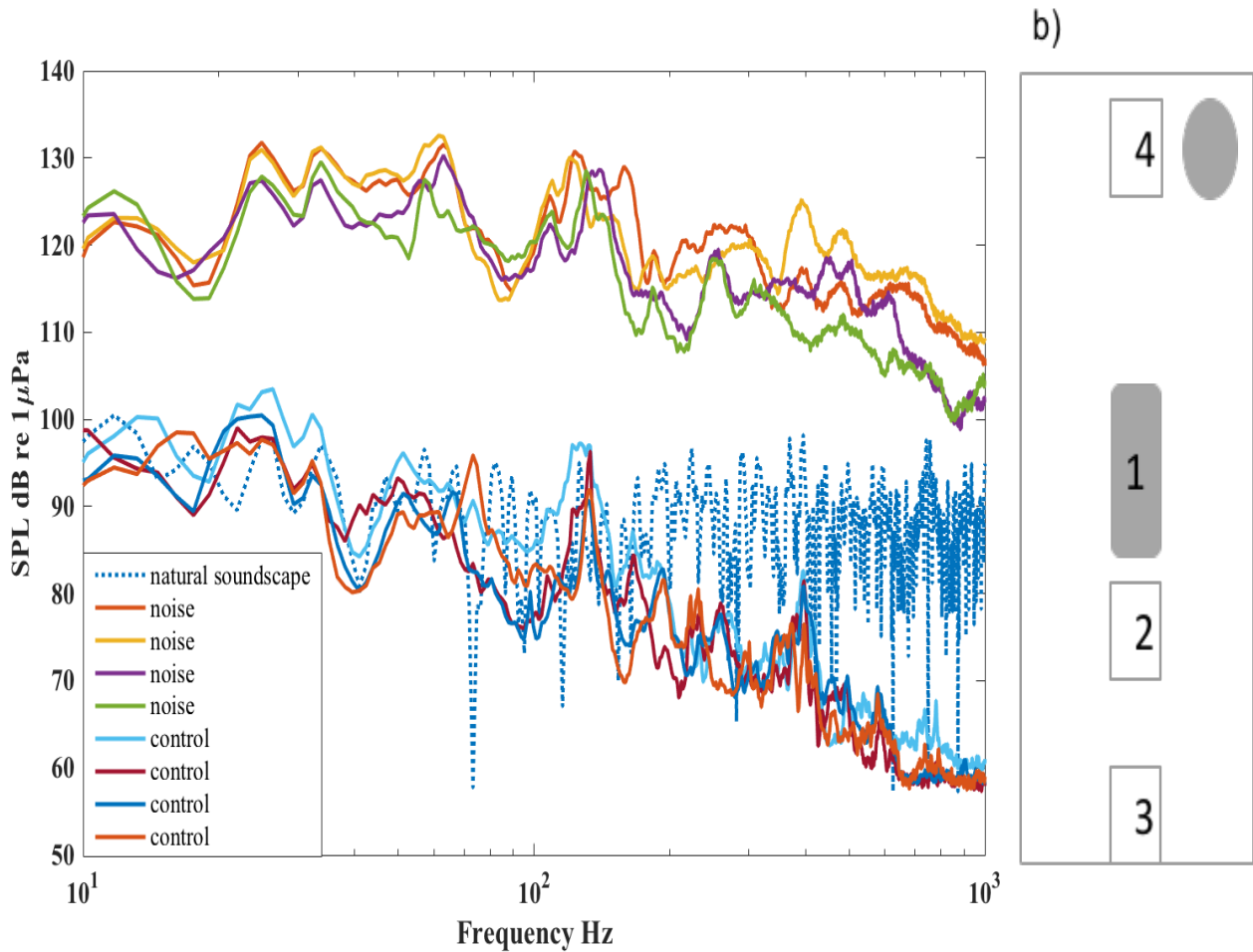


Figure 2. Assessment of noise output in the aquaria, used in paper IV.

(a) Power spectra for noise and control treatments shown for 1-2 kHz. Sound pressure level was on average 34 dB higher for noise than for control for the 1-2 kHz frequency range (36 dB for 0-12 kHz). Natural soundscape is recording from the bay where the fish was caught. (b) Map of sound measurements within the aquaria. Before the fish were placed in the aquaria, noise levels were measured by a hydrophone, placed at four different locations in the experimental aquaria: Location 1 - inside the nest, location 2 - 10 cm in front of the nest, location 3 - 20 cm in front of the nest and location 4 is 10 cm behind the nest, near the sound source (marked as a round grey circle).

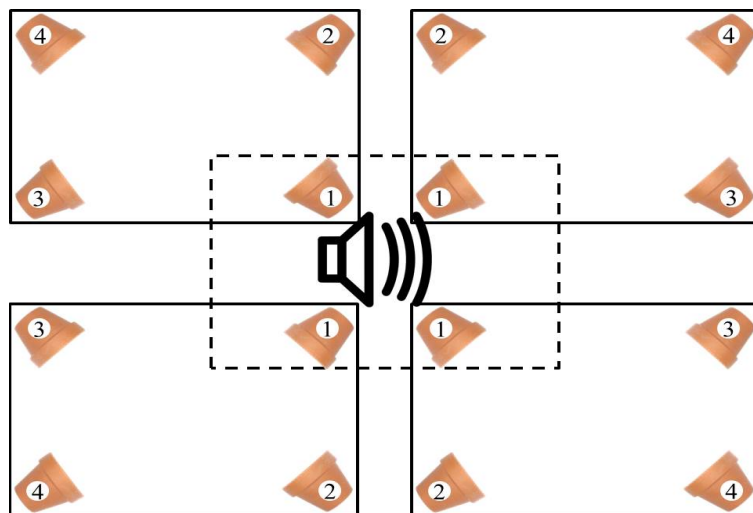


Figure 3. Set-up in **paper V** seen from above, to test the effect of noise on sexual selection in *P. minutus*. Boxes were placed in groups of four with a noise source on top. Each box contained four nest sites, four males and four females.

Lipid analysis

Fish from **paper III** and **V** were analysed for lipid content to estimate body condition. Defrosted fish were dried in a desiccation oven at 72°C for 36 hours before first weight was noted by an accuracy of 0.001 mg by using a microbalance (model XR 205SM-DR, Precisa Instruments Inc., Switzerland). To extract the lipids, each individual was placed in a small glass vial filled with petroleum ether for 12 hours. After removing the fish from the vial, it was left in a fume cupboard for at least 2 hours for the petroleum ether to evaporate. Then the fish was again dried overnight at 72°C in the desiccation oven before taking a second weight measurement. The difference in body mass was used to estimate the lipid content.

Behavioural recordings and analysis

All behavioural recordings were made with a camcorder (Canon Legria HF M56, Ōta, Tokyo, Japan) placed in front of the aquarium at a 90-cm distance. The behaviour was analysed from videos using the event recorder JWatcher, in which each behaviour of either male or female was scored and a time estimate was recorded as an outcome.

RESULTS AND DISCUSSION

Male visual courtship behaviour

I found that male courtship behaviour is similar in both *P. microps* and *P. minutus* (**paper I**). For both species, the male courtship normally starts using fast approaches with erected fins towards females. Typically, the males swim back to the nest in a conspicuous manner, considered a lead display ('lead swim'). Females may choose to follow the courting male into his nest, where the male then often continues his courting with 'displacement fanning' (fanning in the nest in the absence of eggs). An additional observation that turned out to be significant is that during courtship, the short distance movements of *P. microps* were faster than those of *P. minutus*.

Independent of treatment (food treatment or noise), males displayed with the same effort (**paper III, IV**) towards the females and there were no difference in display effort between successful males that received eggs and males that did not receive eggs. Therefore, visual display does probably not reflect on male condition in female mate choice. It might be an advertisement from a male to call on female attention (Számadó 2015) and the female might assess the male condition on another cue. Nest building however, in contrast to visual display, significantly increased with lipid content (condition) of the male and the nest had more sand cover and smaller nest openings i.e. a higher nest quality (**paper III**)(Olsson et al. 2009). Therefore, nest building might be included in one of the multiple cues that a female uses to assess a male in mate choice. Other studies have found that females prefer to spawn in more well-covered nests (Jones & Reynolds 1999; Svensson & Kvarnemo 2005). Since nest building is correlated with condition it should be an honest signal for *P. minutus* females about male condition (but see Lehtonen & Wong 2009).

Female visual courtship behaviour

Female courtship behaviour is similar in both *P. microps* and *P. minutus* (**paper I**). Females of both species present their bellies during courtship, by hopping up and down in small movements in direct proximity to the male.

Both species have facial lines that are highly conspicuous during courtship, though more pronounced in *P. microps* than in *P. minutus*. In *P. minutus*, the eye and the area around the eye turns black (termed ‘dark eye’, **paper II**) during courtship (**paper I and II**). This black coloration is absent in *P. microps* females (Table 1).

Round females, carrying mature eggs, were more likely to show dark eyes than slimmer females (Figure 4, **paper II**) and that all females that spawned during the video recordings had dark eyes. Hence, the dark eyed females may have attracted the male’s attention, but for some reason the males did not respond behaviourally, at least not in a way that I could quantify. This is noteworthy given that a darkening of fins and body is a conspicuous aspect of aggression in male sand gobies (Forsgren 1999) but I could not find evidence that dark eyes play any part in female-female aggression. Since dark eyes are associated with impending spawning, it may be an aspect of female mate choice, possibly a display intended to attract the attention of the male (without necessarily being successful). In contrast to traits indicating aspects of mate quality (Kvarnemo & Forsgren 2000), attention seeking display does not necessarily carry costs or correlate with the quality of the signaller (Számadó 2015).

The display of dark eyes might be unrelated to communication, it may instead be related to female vision. Eye colour has been suggested to act as an anti-glare, and therefore dark eyes could suggest improved accuracy of visual assessment. This phenomenon appears to have received most attention in mammals, and is open to speculation whether it is equally applicable to fish. If applicable, the timing of the dark eyes display found in **paper II**, just prior to spawning or by very round females presumably close to spawning, suggests that such improved vision is likely to be associated with mate evaluation. Given that sand gobies normally adjust their body colour, including eye colour, to the background for camouflage (DeBroff & Pakk 2003), the dark eyes in females appear conspicuous and the temporary nature of the coloration may be an adaptation to ameliorate an otherwise heightened predation risk. Furthermore, most breeding takes place in shallow and sandy bays, glare is a likely environmental condition. It is therefore possible that the dark eyes aid ready-to-spawn females in their assessment of potential mates, although this is a preliminary finding and requires additional research and investigation to validate this function.

Table 1. Synthesis of previously described and novel observations of male and female courtship behaviours of *P. microps* and *P. minutus*

Courtship trait	Ethogram/ Description	Result from present study. Properties, range, median (s) for behaviors				Selected references
		<i>P. microps</i>	<i>P. minutus</i>	<i>P. microps</i>	<i>P. minutus</i>	
Female						
Bobbing & Belly display	Up and down bobbing. Display of belly towards the male	Present	Present	1-1, 3, n=7	1-18, 8, n=3	C.G: (Borg et al. 2002; Nyman 1953) SG: (Fonds 1973)
Black eyes during courtship	Conspicuous black eyes and lines around the eyes of ready-to-mate females	Absent	Present		60-2340, 403, n=5	SG: (Forsgren 1997; Kvammen et al. 1995)
Male						
General male nuptial color	Bands on the sides of the body, anal fin with black edge, a black and blue spot in the first dorsal fin	Present	Present	n=10	n=10	C.G: (Kullander et al. 2012) SG: (Fonds 1973; Kullander et al. 2012)
Blue anal fin		Absent	Present	n=10	n=10	C.G: (Kullander et al. 2012) SG: (Kullander et al. 2012)
Male throat color		Brown-red	Grey-white	n=10	n=10	C.G: (Kullander et al. 2012) SG: (Kullander et al. 2012)
Fin display	Erecting all fins while displaying towards female (when still as well as swimming)	Present	Present	<1-84, 8, n=8	<1-22, 3, n=5	C.G: (Magnhagen 1998) SG: (Forsgren 1997; Forsgren et al. 1996)
Jumps	Short distance fast movements	Present	Present	1-16, 2, n=8	1-2, 2, n=3	C.G: (Jones and Reynolds 1999) SG: (Fonds 1973)
Swim speed	Swimming speed in vicinity of female	13.1 ± 9.1 cm/s	4.3 ± 1.5 cm/s	5-26, 8, n=6	2-64, n=6	Present study
Attack	Fast swimming towards the female including bites	Present	Present	<1-1, 2, n=5	<1-4, 1, n=6	C.G: (Magnhagen 1998) SG: (Fonds 1973)
Lead swim	Targeted swimming towards the nest during courtship context	Present	Present	<1-6, 2, n=3	2-7, 3, n=5	C.G: (Magnhagen 1998; Nyman 1953) SG: (Fonds 1973; Forsgren 1997)
Displacement fanning	Fanning inside the nest also without eggs	Present	Present	1-84, 8, n=7	2-44, 7, n=3	C.G: (Jones and Reynolds 1999) SG: (Forsgren et al. 1996; Pampoule et al. 2004)
Nest building	Nest building during courtship context	Present	Present	<1, 12, 7, n=5	1-19, 4, n=5	SG: (Svensson and Kvammen 2005)
Pre-spawning sound, female in nest	Low-frequency sound in trains of pulses	Present	Present	n=5	n=1	C.G: (Bolgan et al. 2013) SG: (Lindström and Lugh 2000; Pedrosa et al. 2013)
Pre-spawning sound, female outside nest	Low-frequency sound in trains of pulses	Not recorded	Present		n=4	Present study

Range and median (s) are calculated from all recorded behaviors from all individuals that showed the behavior. n number of replicates with an individual showing the trait, CG: *P. microps* (common goby), SG: *P. minutus* (sand goby). Two courtship sounds was analyzed per male i.e. 10 sounds per species

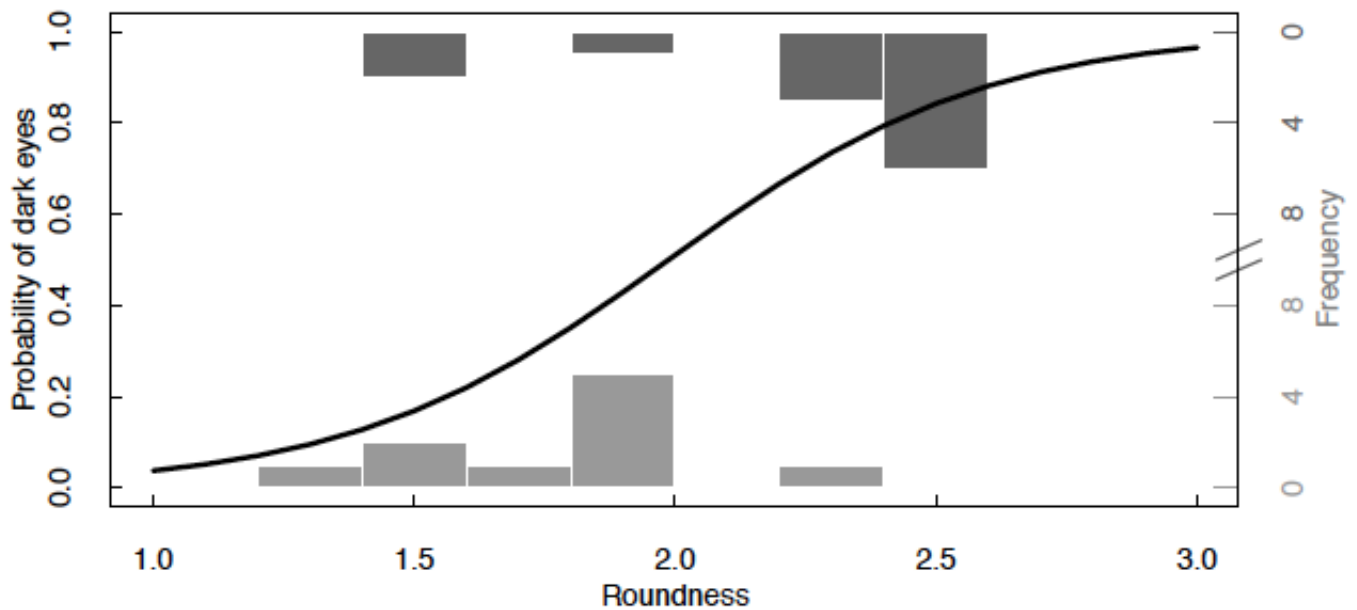


Figure 4. Bar chart illustrating the relation between female roundness (black curve, logistic regression of female roundness and dark eyes) and frequency of dark eyes in replicates where dark eyes were observed (dark grey bars: Females showing dark eyes N = 12, light grey bars: Females not showing dark eyes N = 10).

Acoustic courtship

Another courtship trait found in both males of *P. microps* (**paper I**) and *P. minutus* (**paper I** and **III**) is sound produced during female attraction. Males of both *P. microps* and *P. minutus* produced sound inside the nest when accompanied by a female. Another result is that male *P. minutus* also produces courtship sounds while he is laying in the nest opening and the female is close outside, which has not previously been reported. This is also easy to see visually since the male sticks his head out and starts to quiver. The acoustic displays differ between the species. Males of *P. minutus* sound like a cat purring, while the sound of male *P. microps* rather have the characteristics of a woodpecker. The males of *P. minutus* also produce sound for a longer time, with significantly longer train duration than *P. microps*. The longest sound duration recorded for a male of *P. minutus* lasted 12 seconds. The courtship sound of *P. microps* most likely is ‘louder’ than the

sound of *P. minutus*. Although our study suffers from low sample size and methodological issues, I show that there are clear species differences in sound production (table 2, figure 5).

Species differences in courtship sounds, and other traits involved in mate choice may have diverged as a response to differences in the physical environment as well as intra- and inter-specific interactions (Gröning & Hochkirch 2008; K. S. Pfennig & D. W. Pfennig 2009; Bradbury & Vehrencamp 2011; Wilkins et al. 2013). A difference in acoustic signal might also function as a cue to choose the right species. Because mate choice takes time and prolonged conspicuous courtship increases exposure to predators (Magnhagen 1990; Magnhagen 1991) both males and females should be selected to avoid spending time on inspecting and courting the wrong species.

An observation is that when males of both *P. microps* and *P. minutus* produce sound they quiver. Could it be that the male quivers to stimulate the female to lay eggs and that the low frequency sound is just a by-product of this? Both *P. microps* and *P. minutus* have a fused pelvic fin that they use when sitting on a substrate, and can this pelvic fin have a function to sense ground borne vibrations? If this is plausible, then the sound added to the aquaria in **paper IV** and **V** caused vibrations that may have made it harder for the female to sense the sounds produced by the male.

Table 2. Acoustic features from males of *P. Microps* and *P. Minutus* produced during courtship

Acoustic parameters	<i>P. microps</i>			<i>P. minutus</i>			Permu-tation test
	Median	Range	Range (abs)	Median	Range	Range (abs)	
Number of pulses per train	15.0	11.5–17.5	9.0–18.0	32.5	13.0–89.0	10.0–121.0	<i>p</i> = 0.055
Train duration (ms)	535	370–390	280–840	1060	625–3450	400–4900	<i>p</i> = 0.040
Pulse repetition rate (pulse/s)	26.8	22.2–37.9	21.4–46.7	26.1	20.5–29.5	18.0–31.4	<i>p</i> = 0.41
Pulse period (ms) between two pulses	39.9	38.4–45.1	36.7–46.7	40.0	33.3–50.9	30.0–55.0	<i>p</i> = 0.95

Descriptive statistics is based on the median of the two first pulse trains in one burst produced by each male (*P. microps* *n* = 5 males, *P. minutus* *n* = 5 males). Range is the range of the means of the two analyses sounds from each male (*n* = 5 for each species) and range (abs) is the range of all measured sounds (*n* = 10 for each species). Significant differences (at alpha = 0.05) are presented in bold

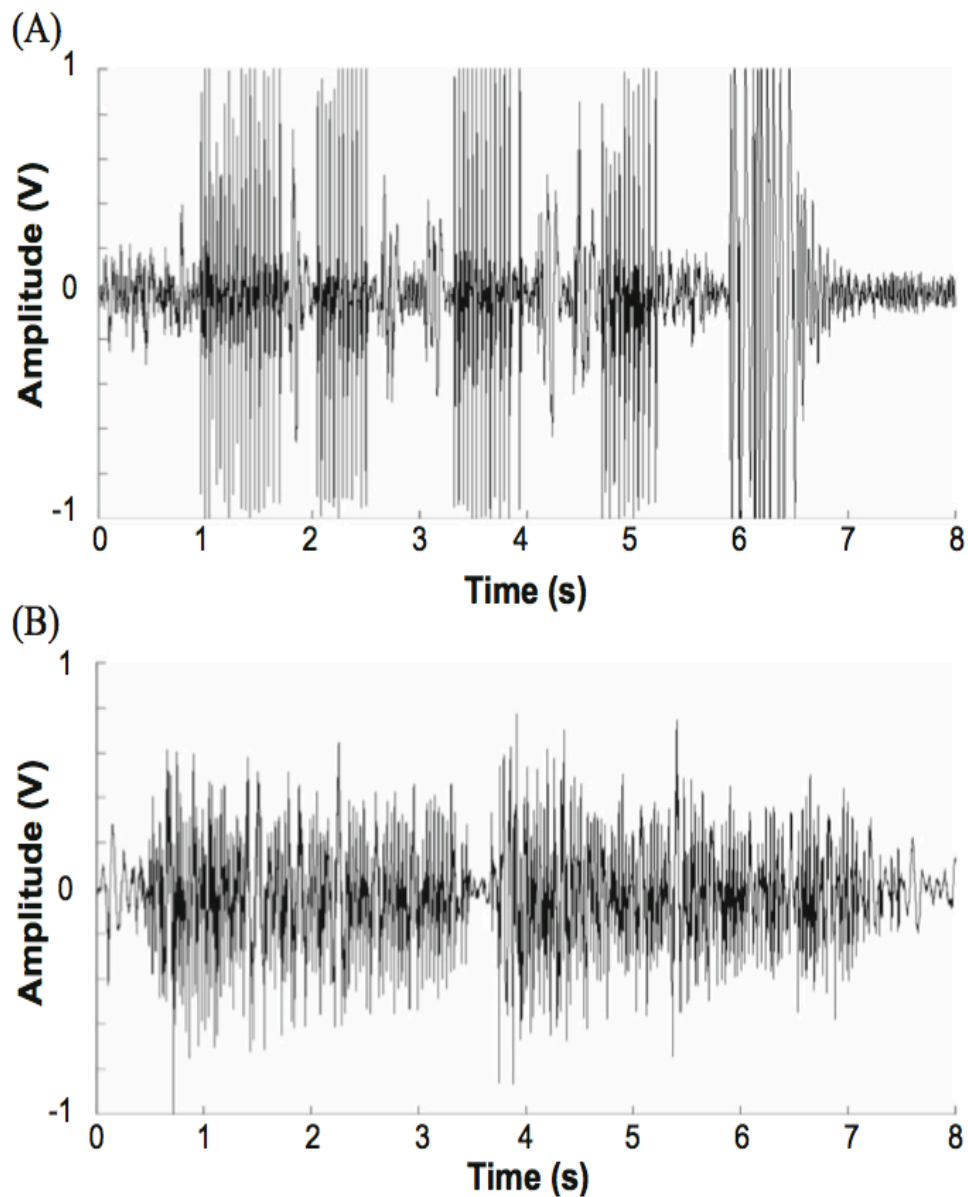


Figure 5. A) The figure shows a representative sound of five trains clustered into one burst produced by a common goby *Pomatoschistus microps* male. Amplitudes are clipped at 1 V. b) The figure shows a representative sound of two trains clustered into one burst produced by a sand goby *Pomatoschistus minutus* male. Both oscillogram were made in Matlab R2009b (The Mathworks Inc., Natick, Massachusetts, USA)

Acoustic display is important in female mate choice

Females only spawned with males if they produced an acoustic courtship and therefore, it is possible that sound is an honest signal to the female (**paper III**). This is consistent with previous studies in fish where it has been shown that the maximum calling rate of male damselfish are positively correlated with brood size, suggesting that a high calling rate is selected since it reflects on male condition (Mann & Lobel 1995). Similarly, the female of Lusitanian toadfish (*Halobatrachus didactylus*) can assess male condition by vocal activity and mating call characteristics (Amorim et al. 2010).

Noise affects sexual selection

I further provide evidence that broadband noise with most energy below 1 kHz affects reproductive success in *P. microps* and *P. minutus* (**paper IV and V**). In **paper IV**, and when exposed to continuous noise, fewer pairs spawned (figure 7 a) and males remained unmated for a longer time (figure 6) despite male behaviour remaining apparently unaffected. In (**paper V**), the silent treatment the most successful males (in terms of number of eggs in the nest) were significantly larger and in better condition than unsuccessful or less successful males. In contrast, in the noise treatment, successful and unsuccessful males did not differ in size of condition.

It is possible that the females in **paper IV** were more affected by noise and therefore less likely to spawn. In contrast with males in the same study that had a previous noise exposure for 36 h and therefore might have become habituated, noise was novel to the females except for the 1 h of acclimation, possibly causing stronger stress responses. In addition, noise very likely hampered the acoustic signals from the males (**paper IV** and possibly **V**). This suggests that disturbance caused by noise can influence mating decisions and traits under sexual selection in *P. microps* and *P. minutus*.

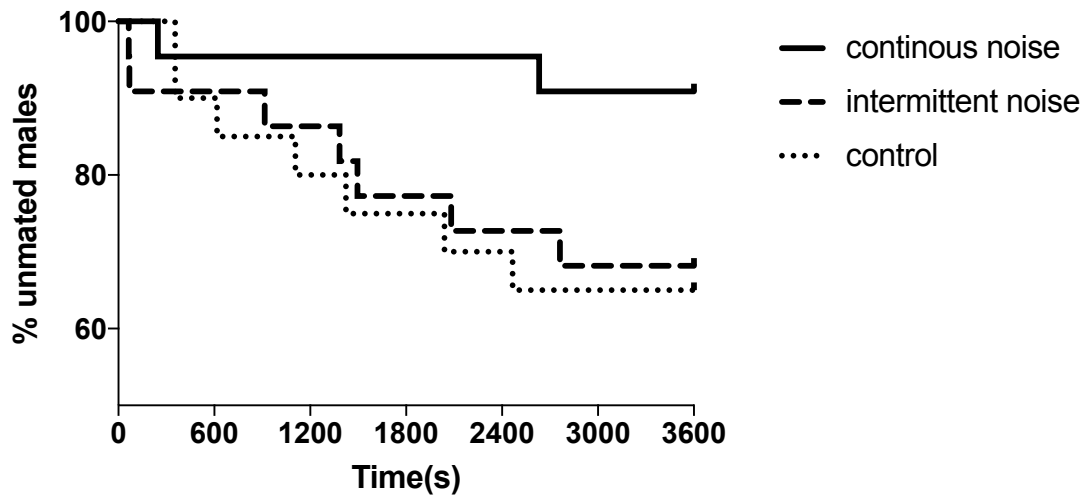


Figure 6. Time to spawn is significantly affected by continuous noise but not intermittent noise

Effect of treatment (control, intermittent and continuous noise) on ‘time to spawning’ in the common goby (*Pomatoschistus microps*). Kaplan-Meier survival curves show the percentage of unmated males over time. Pairs spawned significantly faster in the control than pairs in the continuous noise exposure ($p = 0.04$).

Noise effect on hatching

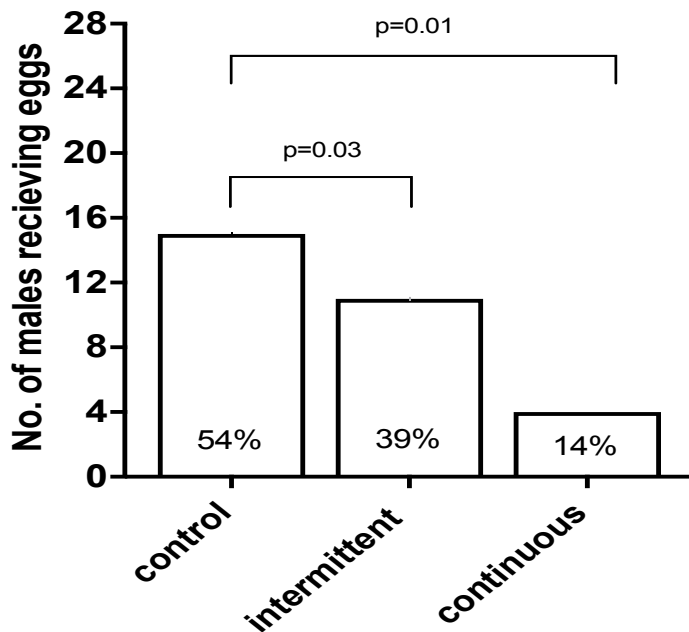
In **paper IV**, clutches hatched earlier in the control treatment than in both noise treatments. There are several potential explanations to this result. First, as clutches in the noise treatments were more dense (figure 7 b-c) than in the control treatment it is possible that the oxygen supply decreased around the eggs (Green et al. 2006). Oxygen deficiency is known to reduce the developmental rate of fertilised eggs (Einum et al. 2002; Lissåker et al. 2003; Rombough 2007). Secondly, recent laboratory studies show negative effects of noise on fish larval development (Nedelec et al. 2015), so the effect might have been directly on the embryos. Finally, a decrease in egg development rate could be explained by a decrease in male parental care, that for example was due to elevated cortisol levels induced by noise exposure (Nichols et al. 2015). Regardless of which explanation is more accurate, in a fish with a single short breeding season, a decrease in development rate has the potential to negatively affect lifetime reproductive success. Therefore, our results show a potentially important impact on individual fitness.

Figure 7 a-c

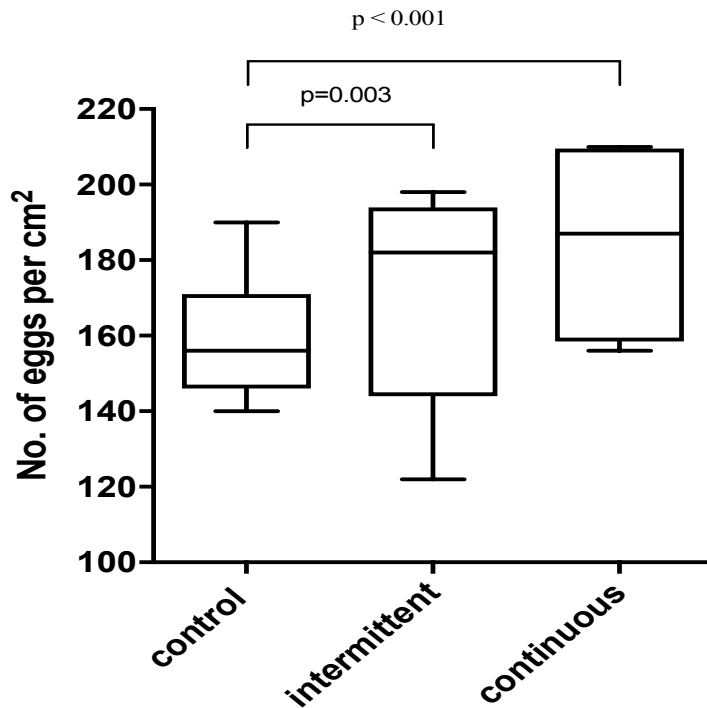
Noise treatment affects the reproductive outcome of the common goby

Comparisons of the effect of treatment (control, intermittent and continuous noise) on different aspects of reproductive outcome in the common goby (*Pomatoschistus microps*).

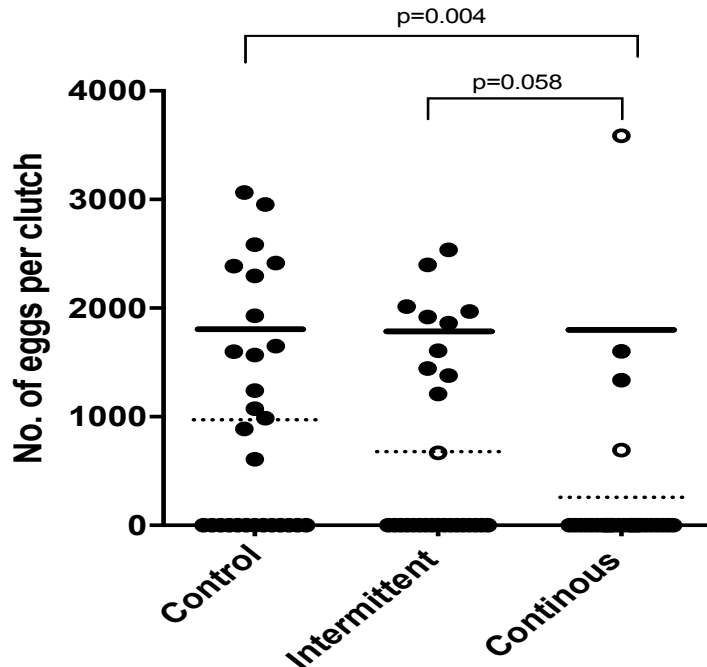
a)



(a) Bars show male mating success, measured as occurrence of spawning. Out of 28 males per treatment, 15, 11, and 4 males from respectively the control, intermittent noise and continuous noise treatments received eggs. Mating success, measured as percent males receiving eggs, is given at the base of each bar.



(b) The boxplot show number of eggs per cm² for spawning pairs, medians, 25th, 75th percentiles, whiskers show the 95th percentiles with outliers.



(c) The aligned dot plot shows number of eggs per clutch. Dotted line shows mean reproductive success of all males, including those 54 males that did not receive eggs and the 3 males that ate the full clutch (n=84). Black line shows mean number of eggs per clutch (n=30). The three clutches that were eaten are marked with a clear scatted dot.

CONCLUSIONS

Investigating the spawning behaviour and the associated acoustic courtship of *P. microps* and *P. minutus* I have produced new knowledge that will help in understanding how these behaviours are affected by anthropogenic noise with applications to wild populations as well as how it might affect results collected in noisy experimental situations. By comparing the two species of *Pomatoschistus*, I highlight differences in courtship behaviours and signals such as train duration of courtship sounds. These differences may be consequential for how specific species react to a changing environment, including species identification and reproductive interference. Furthermore, dark eyes in females of *P. minutus* are associated with readiness to spawn, but the exact function is not fully understood and it seems to be unrelated to male mate choice. These black eyes are absent in *P. microps* females.

It might be an aspect of female mate choice, displayed as part of female courtship behaviour towards the male. Alternatively, it may have a completely different function, possibly related to vision. An interesting study by DeBroff (2003) (DeBroff & Pakk 2003) where 46 students were tested for contrast sensitivity, showed a significant difference between a control and a group who had black grease around the eyes. And their suggestion that when exposed to sunlight, the contrast sensitivity can improve since black grease reduces glare might be plausible with our result. Males of *P. minutus* have a blue spot on the anterior dorsal fin and a blue and black band on the anal fin, which is strongly coloured during a courtship attempt – and the blue colour is absent in *P. microps* males. The female might, by darkened eyes, increase the contrast sensitivity to the male ornament. If this is true it also might affect outcomes when conducting experiments in aquaria in room without natural daylight since important cues might be hampered.

Even if visual display is a large part of male courtship, acoustic display produced by the male in a reproductive context is an absolutely crucial trait for *P. minutus* to receive eggs from a female. This trait was also not affected by lipid content which reflects on male condition. Due to low sample size, it was not possible to conclude if there are any differences in the acoustic signal between males who received eggs and not, but that would be highly interesting to investigate in further studies. Since acoustic courtship is an important trait in male mate choice, disturbance by noise can have a broader

impact on teleost fishes than previously appreciated (Holles et al. 2013; Nedelec et al. 2015; Simpson et al. 2016; Neo et al. 2016), affecting the reproductive success of adult fish. The effects on *P. minutus* were seen already at a small increase of noise level.

Males with the highest geometric mean of length and condition namely the ‘Schwarzenegger index’ were the most successful ‘silent’ control treatment with only ambient noise whereas this was not the case in the treatment with increased noise levels. More males per replicate received eggs in the control compared to the noise treatment, but there was no significant effect of noise treatment on the number of eggs spawned (**paper V**). In contrast in **paper IV**, when experimentally testing an increase of noise levels on *P. microps*, the egg density increased significantly and the eggs took longer time to hatch compared to a control with ambient noise, whereas male nest-building behaviour and active pre-spawning behaviour (including courtship) were unaffected. Reluctance to spawn also increased with noise exposure; fewer pairs spawned in the continuous noise treatments compared to the control, and those pairs that did spawn did it later in the continuous noise treatment than in the control.

Despite the fact that the added noise used in our studies was broadband and of similar frequency interval as boat noise (Nichols et al. 2015; Simpson et al. 2016) with most energy below 1 kHz, it is important to note that noise experiments carried out in aquaria cannot reliably mimic exposure to real boat noise in nature. The acoustic field is more complex and particle motion, a component in sound waves that fish and invertebrates is sensitive to (Nedelec et al. 2016), occurs in a more complex pattern in aquaria than in the open sea (Parvulescu 1967).

Taken together, in this thesis regarding aquatic noise, I show that noise negatively affects reproductive success, highlighting its potential to impact fish demography. Future studies in natural conditions are required for a better understanding of the impact of noise on fish reproduction and possibly also offspring development. Although my findings should not be directly extrapolated to fitness consequences in nature, they represent new and important evidence of the impact of noise exposure on fish reproductive success and highlights the need to examine the effects of man-made noise on fish behaviour and reproduction.

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or who to blame...

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2 grams. This in turn give a kilo price of 107 143 Euros. With this in mind, use another forecast service before going abroad! All the people at **Sven Loven Kristineberg**, you are too many to name but *all* of you are amazing and you really made field work go smooth and easy. Thanks also to all co-authors not mentioned here.

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