



The impact of wave disturbance on the nesting  
behaviour of the sand goby, *Pomatoschistus minutus*

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Master's Thesis

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Nesting behaviour is a form of parental care; nests are often used to ensure the survival of eggs and offspring and to protect them from environmental threats. Sand goby, *Pomatoschistus minutus*, males build nests under any suitable substrate, such as the shell of a bivalve or a stone, in a soft-bottom littoral area. Nest building is dependent on many factors including the cost to parents. I studied the impact of wave disturbance on the nesting behaviour of male sand gobies using wave tanks to simulate different wave velocities. Treatment tanks were set to velocities between 8 cm/s and 18 cm/s whilst control tanks had no wave action. A male was placed in each tank for 23 hours with a choice of a smaller 6 cm x 6 cm tile or a larger 10 cm x 10 cm tile as nesting sites. Whether a nest was built as well as the choice of nest site was recorded. Although binomial generalised linear models showed no effect of wave velocity on the nesting behaviour, males showed more tendency to build a nest in treatment tanks with lower wave velocities but also more tendency to choose to build a nest at a larger tile in higher velocities. I postulate that it could be more beneficial for a male to build a nest at larger site in higher wave disturbances and, therefore, only males that are capable of building a larger nest will build any nest as a smaller nest is not beneficial enough. Wave disturbances may have a larger effect on nest building at higher velocities, but more research is needed.

Keywords: sand goby, nesting behaviour, nest building, wave disturbance, wave velocity.

## Swedish summary

### *Påverkan av vågstörningar på sandstubbens, Pomatoschistus minutus, bobyggebeteende*

Bobygge är ett viktigt beteende för många djurarter för att försäkra överlevnaden av deras ägg och avkomma samt för att skydda dem från miljöhot. Beteende kan trots det vara en kostnad för föräldrarna eller för deras framtida parningsframgång. Man tror också att bon kan vara viktiga vid val av partner och man känner till att vissa fiskarter lägger till ornament i konstruktionen, möjligtvis för att attrahera en partner.

Sandstubben, *Pomatoschistus minutus*, är en fiskart som är vanlig i Östersjön. Hanen bygger bo under ett lämpligt substrat såsom ett snäckskal eller en sten och tar sedan hand om äggen genom att fläktas för att tillföra syre. Dessutom skyddar hanen äggen från yttre faktorer. Därför är valet av substrat beroende av många faktorer såsom temperatur, exponering och predatorer. Trots att andra sandstubbar är ett av de vanligaste hoten mot sandstubbbon och sandstubbägg är de inte det enda hotet. Vågor och havsströmmar kan förorsaka mycket skada på bon och man tror att boområdet delvis väljs för att skydda från vågexponering. Östersjömodeller har visat att det kan förekomma större stormsvallvågor i framtiden. Detta kan innebära att sandstubbar, och andra Östersjöarter, kommer att vara tvungna att anpassa sitt beteende. Hot av alla slag kan leda till att kostnaden för bobygge ökar och att hanarnas kondition minskar. Eftersom hanarnas bobygge är viktigt för sandstubbens reproduktion är det viktigt att veta hur vågstörningar påverkar denna beteendeegenskap.

I detta projekt har jag studerat vågors effekt på sandstubbshanens bobygge och på vilken storleks substrat som väljs för byggandet av boet. Projektet utfördes vid Skärgårdscentret Korpoström i juni 2020. Fyra vågtankar användes för att simulera våghastigheterna. Tankarna är 3,0 meter x 0,5 meter i storlek. Två av tankarna användes för behandlingar och två som kontroll. Behandlingstankarna justerades för att producera jämna vågor som varierade mellan 8 och 18 centimeter per sekund (cm/s). I varje tank placerades ett 6 cm x 6 cm och ett 10 cm x 10 cm kakel som fungerade som substrat för bobygget.

Min hypotes var att hanar i kontrolltankarna skulle bygga flera bon och välja det större substratet på grund av avsaknad av konkurrens från andra hanar. Jag

förväntade mig att hanarna skulle bygga färre bon i behandlingstankarna och att de som byggde skulle välja det mindre kaklet eftersom mera energi kan behövas för byggande i högre exponering. Totalt användes 48 hanar i 48 experiment.

Binomiala generaliserade lineära modeller (eng. *generalised linear model*) visade inget samband mellan våghastighet och bobygge, men hanarna hade en större tendens att överhuvudtaget bygga bo i lägre våghastigheter än i högre. En annan binomial generaliserad lineär modellanalys visade inget samband mellan våghastighet och val av kakel. Däremot fanns det en tendens till att sandstubbshanarna med större sannolikhet valde det större 10 cm x 10 cm kaklet i högre våghastigheter jämfört med i de lägre hastigheterna. En kovariansanalys visade att bona tenderade att ha bredare boöppningar i de högre våghastigheterna. En del av resultaten från detta experiment stämde inte överens med tidigare studier, och detta kan delvis bero på att vågtankarna är miljöer som orsakar mera stress.

Fastän våghastigheter inte hade någon effekt på sandstubbens beteende vid bobygge hade hanarna i behandlingarna en större tendens att bygga bo i lägre våghastigheter. Det här var i enlighet med min hypotes. Samtidigt byggde hanarna i de högre våghastigheterna med större sannolikhet bo vid det större 10 cm x 10 cm kakel, vilket inte överensstämde med min hypotes. Detta kan bero på att större bosubstrat är mera stabila vid högre exponering. Avkomman har också högre framgång i större bon och det är möjligt att det är mera förmånligt för hanar att bygga bo under större kakel i högre vågstörningar, även om själva kostnaden för hanen är högre. Man kan då tänka sig att endast hanar som är kapabla att bygga större bon vid högre exponering bygger bon, då ett mindre bo inte är tillräckligt fördelaktigt. Att boöppningarna var bredare i högre våghastigheter kan bero på att det behövs mindre fläktande för att föra in syre i ett bo med större naturlig ventilation.

Sammanfattningsvis fann jag inte att våghastigheter mellan 8 cm/s och 18 cm/s är ett signifikant hot mot bobyggebeteende men det fanns trender som visade att högre hastigheter kan leda till förändringar i beteendet.

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## 1. Introduction

Many species invest in future generations to help with the survival of offspring, despite costs to the parents and to future mating success (Gross 2005, Pampoulie et al. 2004). This form of parental investment increases the reproductive success of the parent (Gross and Sargent 1985). Parental care is thought to have evolved as a result of immediate fitness benefits that are gained by the survival of current offspring being favoured before the delayed fitness that is gained through further reproduction (Wade and Shuster 2002). However, parental care will only be provided if the benefits of the care to offspring is greater than the cost to the parent (Royle et al. 2012). In mammals this care usually falls to the female, possibly as a result of the inability to confirm the paternity of offspring, but in species with external fertilisation, such as some fish, it might be that the sex that is closer to the egg during fertilisation becomes the caregiver (Wade and Shuster 2002).

In some taxa, such as mammals and birds, this parental care is invariable in its vitality for the survival of offspring whereas in other taxa, including fish, the vitality of parental care is variable (Kölliker et al. 2012). Parental care is more common in freshwater fish (Gross and Sargent 1985) but when parental care is provided in fish (20% of families), 50% of families provide male-only parental care, 30% female-only care and 20% biparental care (Gross 2005).

Female care in fish is correlated with internal fertilisation, whilst male care is correlated with external fertilisation (Gross and Sargent 1985). This, similarly to mammals, is due to the genetic relatedness to the offspring (Gross and Sargent 1985). In species with internal fertilisation, a male cannot confirm the paternity of offspring, but it is more feasible to confirm the paternity of eggs in species where eggs are fertilised externally. A male, or female, will not weigh the benefits of parental care against the costs if there is not a certainty of genetic relatedness. The dominance of male parental care in fish is hypothesised to have evolved from spawning territories (Werren et al. 1980) and male care has been seen to be associated with males being territorial over spawning areas (Gross and Sargent 1985).

Parental care in fish takes various forms. Most forms of parental care can be provided by either sex, depending on species. The most common form is guarding; 95% of species that provide parental care guard the offspring (Gross and Sargent

1985). A guarding parent chases away threats to the eggs or fry, including both conspecifics and heterospecifics (Blumer 1979). Another form of parental care is fanning, where the parent fans the egg mass or fry with their body to aerate the area and remove sediment (Blumer 1979). Some fish species have internal gestation, where the eggs develop in the ovaries or oviducts and others have brood pouches where the eggs develop in an external sac on one of the parents (Blumer 1979). Eggs may also be held in the mouth or gill chamber during development or attached to the body (Blumer 1979). Eggs and fry may be moved between locations and fry that move away from the nest or school of fry can be retrieved (Blumer 1979). Some species remove dead or diseased eggs from the nest and others clean eggs inside their nesting cavities in their mouths (Blumer 1979). Some species produce a mucus on their skin to feed young fry (Blumer 1979). In more shallow waters, some fish species coil around the offspring to guard them from exposure and low tides, whilst other species splash eggs that are exposed to the air (Blumer 1979). Whilst some species bury their eggs in sediment, others build nests (Blumer 1979).

Nest building is a prime example of nesting behaviour (Bendesky et al. 2017, Barber 2013). Nests are used across many animal taxa (Barber 2013), often to ensure the survival of eggs or offspring and to protect them from environmental threats (Pärssinen et al. 2019). In fish, nests are also thought to be an important factor in mate choice and attraction (Blumer 1979, Barber 2013). Some fish, including sticklebacks, have been shown to add non-bodily ornaments to the constructions (Schaedelin and Taborsky 2009), possibly to attract mates. Previously it was thought that nest building only paid a cost to the males, existing only to further the survival and success of the offspring. However, a study conducted on sand gobies showed that it is possibly a sexually selected trait that increases a male's mating success (Lindström et al. 2006).

The sand goby, *Pomatoschistus minutus* (Pallas 1770), is a littoral fish and common along the European Atlantic coast, including the coasts of the Mediterranean, North Sea and Baltic Sea (Herler et al. 2014). Male sand gobies build nests under any suitable substrate, such as the shell of a bivalve or a stone, by removing sand from underneath the substrate (Lindström 1988, Figure 1). In the Baltic Sea, their breeding season is usually between May and August, the start of which varies locally as it is dependent on high enough water temperatures (Lindström 1988). The choice of nesting site is dependent on a multitude of factors such as temperature, exposure to waves and protection from predators (Lindström 1988). Females will lay eggs in

batches in the nest, in a single layer (Lindström and Hellström 1993). Males prefer to mate with multiple females and females often choose to lay eggs in a nest which already houses eggs (Lindström et al. 2006; Forsgren et al. 1996). Males care for the eggs throughout the entire egg phase by protecting the nest from predators and conspecifics and removing any destroyed eggs or other debris from the nest (Lindström 1988, Lindström and Hellström 1993). They will also perform fanning, which increases the oxygen concentration around the eggs (Lindström 1988, Bjelvenmark and Forsgren 2003). It has previously been shown that male sand gobies in the northern Baltic Sea prefer larger nest sites (Lindström 1988, Flink and Svensson 2015) and nest size is a determinate in male mating success, as larger nests have more space for eggs (Lindström 1988). Larger males are often correlated with larger nesting sites, possibly due to more competition for these preferable sites that larger males are more adapted for (Lindström 1988).



**Figure 1.** In nature, the male sand goby builds a nest under a suitable substrate, in this image a bivalve shell. The female sand goby then lays a batch of eggs in the nest that the male proceeds to care for (photo Aurora Garcia-Berro).

Conspecifics are one of the most common threats to sand goby nests. In many nest-defending fish, including the sand goby, larger individuals may take over nests and resources from smaller males (Lindström 1992, Hamilton 1998). Though nests are thought to be built to protect eggs from predators (Lindström 1988), male sand gobies



do not seem to alter their reproductive behaviour in the proximity of predators. This includes both predators to males and predators to the eggs (Magnhagen and Forsgren 1991, Lehtonen et al. 2013).

Conspecifics are not the only threat to sand goby nests. Currents and wave action can cause much damage to nests, as sand gobies often breed on shallow littoral soft-bottoms where waves often cause major physical disturbance (Figure 2). As a result of this, sand goby nest sites are possibly chosen to protect the nests and eggs from wave action. (Lindström 1988).



**Figure 2.** One of the study sites, Ängsö, from where male sand gobies used for the experiments were caught. Sand gobies often mate in shallow soft-bottom littoral areas, such as the one seen in this image (photo Kai Lindström).

Wave action impacting nests is a common occurrence amongst many fish species. One common risk to nest-building fish in flowing water is losing nests due to water flow (Rushbrook et al. 2010). Studies on river blennies showed that they orient their nests in a direction in which the velocity of the current entering the nest is significantly lower than that of the regular current (Vinyoles et al. 2002). Studies on the five-spotted wrasse found an association between sea condition and nesting attempts in that there were more nesting starts in more favourable sea condition (Raventos 2004). The effects of disturbances of this type have not only been studied in fish species. A study of burrowing owls has shown that they will lessen their nest

defensiveness in harsher wind condition (Fisher et al. 2004); it may be that fish species react in a similar way. Many fish species have been able to adapt to the present wave and current climate, but there may be changes to the intensity and frequency of waves in the future. Models have shown that climate change caused by carbon dioxide emissions may have an impact on wave climate and conditions (Morim et al. 2019, Graham et al. 2007). Whilst global models have shown that the average wave height may decrease by over 25% (Hemer et al. 2013), some models for the Baltic Sea area have shown that a combination of changes to wind patterns and a rise in sea level can lead to larger storm surges as well as an increase in wave height (Gräwe and Burchard 2012, IPCC 2019). However, effects of a change in wave climate on fish has not been the focus of much research in the past.

Threats and stresses typically add to the cost of nesting behaviour and the protection of nests in sand gobies can lead to a decrease in body condition amongst males (Lindström and Hellström 1993). It has even been shown that an increase in costs can lead to a decrease in nesting behaviour, overall, as the costs begin to outweigh the gains (Rushbrook et al. 2010). As nesting behaviour plays an important role in the reproduction of sand gobies and the choice of nest is important for the reproductive success of an individual, it is important to know if change in wave conditions may impact these behaviours. There are no previous studies regarding these issues and so this project aims to fill this knowledge gap.

In the laboratory, I studied the impact of physical disturbance caused by different wave velocities on nesting behaviour. I looked at whether changes in wave velocities impacted whether a male built a nest as well as if it impacted the choice of a smaller or larger nest site. Wave tanks were used to simulate different wave velocities. I hypothesised that males in control tanks without waves should build nests and should choose the larger nest, as there is no competition. This would be in line with previous studies. Fewer nests were expected to be built in tanks with waves and a smaller nesting site should be chosen more often, as more energy could be spent fighting against the current instead of building a nest at a larger site.

## 2. Materials and methods

### 2.1 Collection and housing of sand gobies

This project was conducted at the Archipelago Centre Korpoström in June 2020. Sand goby males used in the project were collected from nearby sites (Fårö and Ängsö) using a beach seine. The males were collected weekly whilst the experiments were underway. The sand gobies were housed in 60 litre aquaria between collection and commencement of the experiment in which that male was used. The housing aquaria had running water from the sea and the gobies were fed frozen Chironomidae larvae during this time. Sediment used in the tanks had also been collected from a nearby site.

### 2.2 Preparation of wave tanks

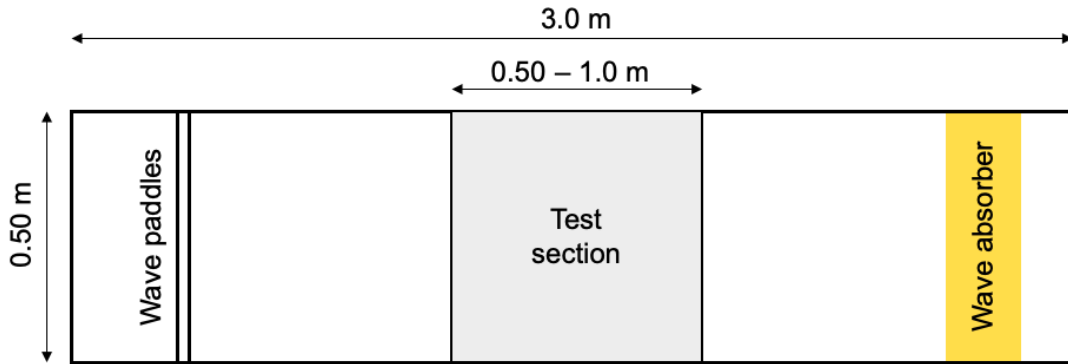
Four wave tanks were used for the project (Figure 3). These wave tanks measured 3.0 x 0.5 metres in size and had an adjustable test section for experiments. It was possible to adjust the pressure, water level ( $\pm 0.5$  cm) and amplitude of the wave paddles in the tanks to create a variation of wave types with different characteristics such as irregularity or regularity of waves, fast or slow wave velocities and high or low wave frequency. There were wave absorbers in each tank to absorb the previous wave to keep the chosen characteristics constant. Figure 4 shows the set-up of the tanks in clearer detail with the wave paddles, test section and wave absorbers marked.

The pressures of the wave mechanics were set at 2 bars in all tanks to secure continual flow during the entirety of the experiments. The amplitudes of the wave paddles were adjusted in two of the tanks to create even, regular waves with wave velocities varying between 8 centimetres per second and 18 centimetres per second (cm/s) with an average of 11.98 cm/s (sd = 2.70 cm/s). These tanks served as treatments. The wave mechanics were turned off in the remaining two tanks, which served as constant controls. Plexiglass was placed on the outer sides of the test section of treatment tanks with wave disturbance to uphold the evenness of the waves; plexiglass was not used in the controls. Sand was used as the bottom substrate in the test section for the experiments and 10 centimetres of sand was used to create an even base throughout the three metres of the tanks for wave flow. The water levels were set at 30 centimetres above bottom level. Water for the tanks was collected from the sea

and run through a sand filtration. LED lights were set upon each test section to standardise light conditions and were lit between 04.00 and 22.00 to simulate daylight. The control and disturbed tanks were placed alternating in case sunlight had an effect. The choice of which tanks were controls and disturbances were otherwise random.



**Figure 3.** The four wave tanks in the aquaria hall at Korpoström. One male was placed in each tank during the experiment and was minimally disturbed.



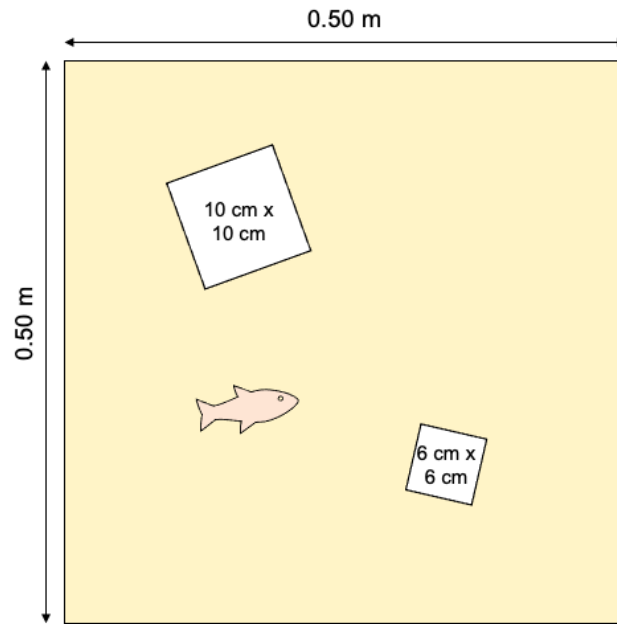
**Figure 4.** Schematic drawing of the wave tank. Waves were created at the wave paddles and moved along the tank towards the test section where the males, as well as the nesting sites, were placed during the experiment. Waves were absorbed by the wave absorbers to ensure that the waves only moved in a single direction (adapted from Marin-Diaz et al. (2020)).

### 2.3 Acoustic doppler velocimeter (ADV)

Wave velocities were measured using an acoustic doppler velocimeter (ADV). Nortek As's ADV Vectrino along with the accompanying computer program was used to measure the wave action. The ADV was placed at 15 cm ( $\pm 0.5$  cm) from the surface of the water and was left to measure wave action for three minutes before the experiment began. The ADV measured ongoing velocity, amplitude and other factors. After measurement the data was exported to MATLAB and the rooted mean square of the horizontal velocity component  $U_{rms}$  was calculated. Hereafter, the  $U_{rms}$  will be referred to as wave velocity.

### 2.4 Experimental design

A 6 cm x 6 cm and a 10 cm x 10 cm white ceramic tile that acted as nesting substrates were randomly placed in the test section of each wave tank, making sure that neither tile was sheltering the other from the disturbing waves (Figure 5). The test sections were adjustable between 0.5 and 1.0 m x 0.5 m but were kept at 0.5 m x 0.5 m for the purpose of the experiment. The sections were separated from the rest of the tank using fine mesh netting. After placement of tiles, the wave mechanics were turned on in the tanks with treatments and the wave velocity was measured using the ADV and adjusted, if needed.



**Figure 5.** Schematic drawing of the experimental design of the 0.5 m x 0.5 m test section. One male sand goby was placed in the sand-bottomed test section along with one 10 cm x 10 cm white ceramic tile and one 6 cm x 6 cm white ceramic tile. The male was left in the test area for 23 hours.

At the beginning of an experiment, one male sand goby was placed in each tank. Before placed in a tank each sand goby male was measured for weight and length. To measure length a digital picture was taken of the fish together with a ruler. Length (millimetres mm,  $\pm 0.1$  mm) was measured in ImageJ. Weight (grams g,  $\pm 0.01$  g) was measured directly on a scale in the laboratory. Gobies varied in size between 34.2 mm and 67.0 mm with a mean of 47.0 mm (sd = 7.6 mm). Fulton's conditional factor was calculated as:

$$\text{Fulton's conditional factor} = 100 \times \frac{\text{weight (g)}}{\text{length (cm)}^3}$$

Fulton's conditional factor shows the relationship between the weight and length of fish and can be used to estimate the physical condition of an individual fish. The conditional factors of the gobies varied between 0.33 and 0.98 with a mean of 0.70 (sd = 0.13). The different-sized males were placed randomly between treatments and controls, with the mean length in controls being 47.4 mm (sd = 8.2 mm, n = 24) and the mean length in treatments being 46.6 mm (sd = 7.0 mm, n = 24). A one-way ANOVA showed that there was no difference in size between control and treatment

( $F_{1.46} = 0.488$ ,  $p = 0.488$ ). There was also no relationship between the order of the replicate and the size of the goby ( $F_{1.46} = 0.119$ ,  $p = 0.732$ ).

In total, 48 males were used in 48 experiments. Half of these replicates were controls and the other half treatments. The gobies were left in tanks for 23 hours with minimal disturbance. After these 23 hours, the nesting behaviour was recorded. Nesting behaviour included the building of a nest, the choice of nesting site and the size of the nest entrance. A digital image of the nest entrance was taken, and the width of the entrance was measured in ImageJ compared to the size of the tile (Figure 6). Gobies were thereafter removed from the tanks and returned to the sea. Thereafter, the tanks were emptied of water and refilled with fresh sea water. The tiles were removed and washed before being used again. Two sets of tiles were used for each tank. This was to ensure that males were not impacted by previous males' choices.



**Figure 6.** The nest entrance was measured in ImageJ. The size of the tile was known and used for calibration. In ImageJ, I compared the known size of the tile to the widest part of the nest entrance to measure the width. The width of the tile is marked in black, and the measured width of the nest entrance is marked in grey.

### *2.5 Statistical analyses*

All statistical analyses were performed in RStudio Version 1.2.5001. The relationship between disturbance and whether a nest was built was tested with a logistic regression (generalised linear model, family = binomial), using the length of the goby as a covariate. The relation between disturbance and tile choice was also tested using a

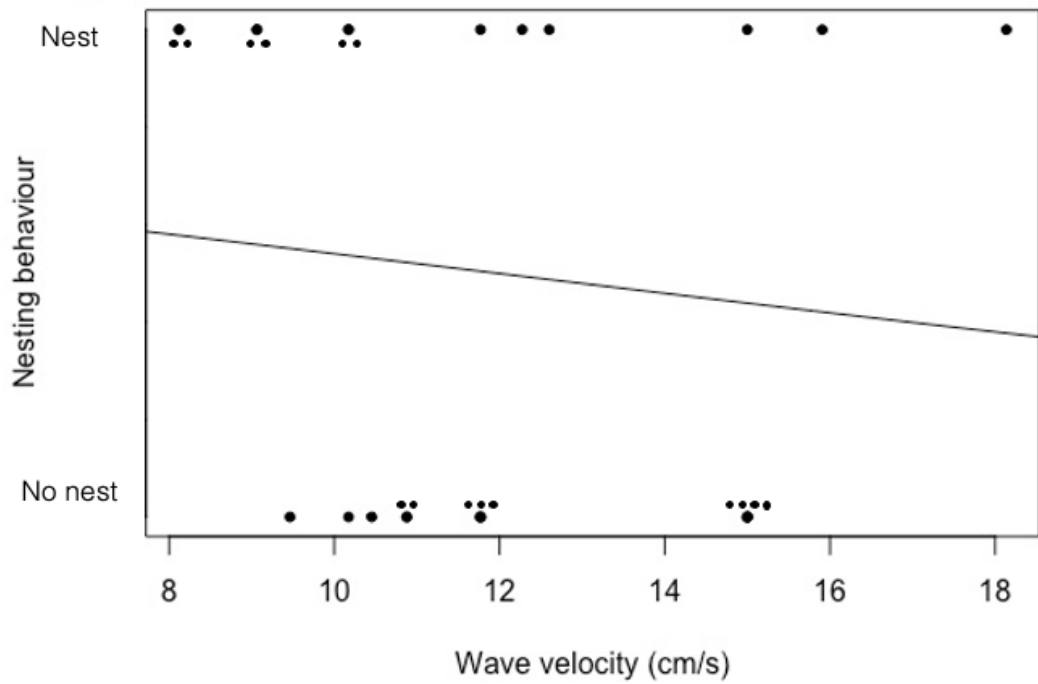
binomial generalised linear model with the length of the goby and Fulton's condition factor as covariates. This analysis excluded the males that had not built a nest. The difference in width of the nest entrance between control and disturbance was tested using an analysis of covariance (ANCOVA), once again using the length of the goby and Fulton's condition factor as covariates.

### **3. Results**

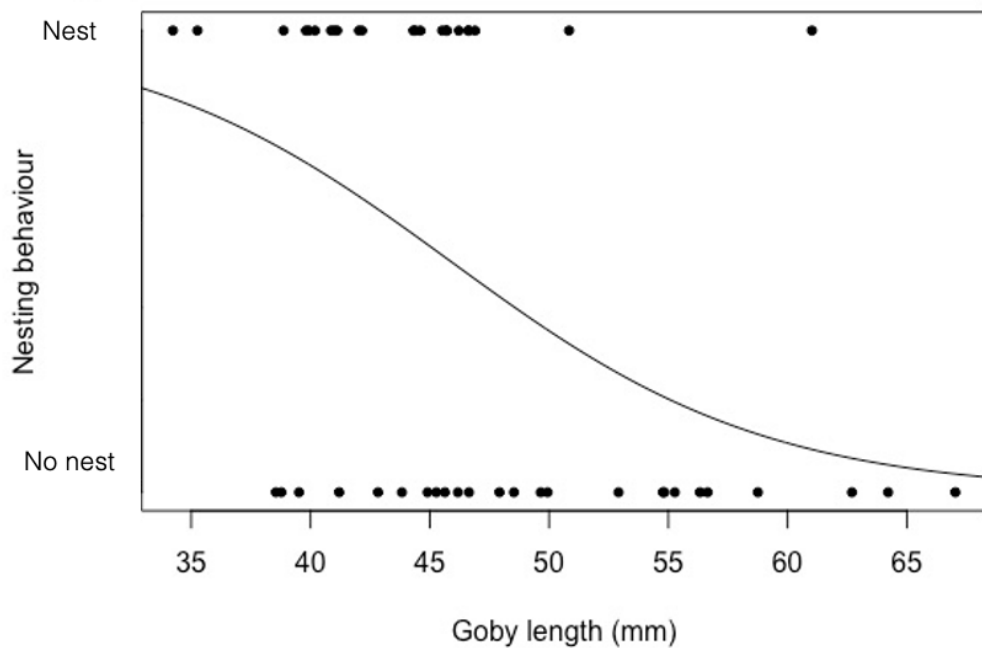
#### *3.1 Impact of wave disturbance on nest building*

Nest build was measured binomially; either the male sand gobies build a nest, or they did not. In controls, 45.8% (n = 11) of males built nests while 50% (n = 12) of males built nests in treatments. Males that built nests were on average 43.5 mm (sd = 5.38 mm, n = 24) and weighed on average 0.613 g (sd= 0.193 g, n = 24). Their Fulton's condition factor was on average 0.734 (sd = 0.102, n = 24). A binomial generalised linear model analysis on all experiments showed no effect of wave velocity on the building of a nest ( $p = 0.695$ , Table 1). However, there was more tendency for males in lower velocities to build a nest than males in higher disturbances (Figure 7). I also found that smaller males were more likely to build a nest in both controls and treatments ( $p = 0.017^*$ , Table 1) (Figure 8). Fulton's condition factor had no effect (df = 44,  $p = 0.658$ ), but there was a tendency towards males with higher condition factors being more likely to build a nest.





**Figure 7.** The impact of wave velocity on nest building. This graph only shows the difference within treatment tanks, not comparing them to control tanks. There was a slight tendency of males in higher wave velocities building less nests than males in lower wave velocities.



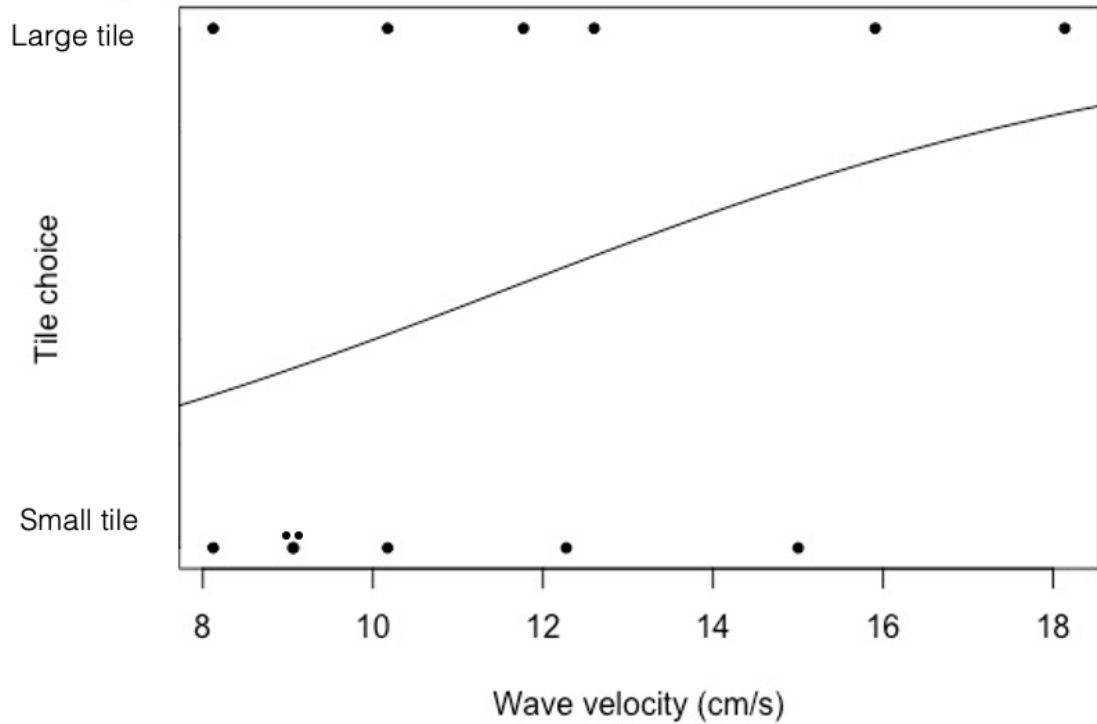
**Figure 8.** The effect of male size on nest building. Smaller males were significantly more likely to build a nest. This goes against previous studies on sand goby nesting behaviour.

**Table 1.** The impact of wave disturbance on nest building was analysed using a binomial generalized linear model. In a logarithmic regression, *estimate* indicates the regression coefficient, *std. error* indicates the standard error, and *z-value* indicates the standard score.

	<b>Estimate</b>	<b>Std. Error</b>	<b>z-value</b>	<b>p-value</b>
<b>Nesting behaviour</b>				
Intercept	5.765	4.166	1.384	0.167
Wave disturbance	-0.021	0.053	-0,393	0.695
Goby length	-0.146	0.061	-2.380	0.017*
Fulton's condition factor	1.413	3.195	0.442	0.658

### 3.2 Impact of wave disturbance on nest choice

Nest choice was analysed binomially amongst the sand gobies that had chosen to build a nest. In total, 54.5% (n = 12) chose to build a nest in a smaller 6 cm x 6 cm tile nest site whereas 45.5% (n = 11) chose the larger 10 cm x 10 cm nest site. In treatments, 50% (n = 6) chose the smaller tiles and 50% (n = 6) chose the larger tiles. Males that chose the smaller tiles were on average 43.27 mm (sd = 7.25 mm, n = 12), weighed 0.605 g (sd = 0.246 g, n = 12) and their Fulton's conditional factor was on average 0.728 (sd = 0.090, n = 12). Males that chose the larger tiles were on average 44.06 mm (sd = 2.62 mm, n = 11), weighed 0.635 g (sd = 0.124 g, n = 11) and had an average Fulton's conditional factor of 0.742 (sd = 0.120, n = 11). A binomial generalised linear model analysis showed no effect of wave velocity on a male's choice of nest size (df = 19, p = 0.613, Table 2), although males showed slightly more tendency of being more likely to choose the larger 10 cm x 10 cm tile as a nesting site in a higher wave disturbance compared to a lower wave velocity (Figure 9). There was no relationship between the length of the goby and nest choice (df = 19, p = 0.585, Table 2) or the goby's Fulton's conditional factor and nest choice (df = 19, p = 0.975. Table 2).



**Figure 9.** The impact of wave velocity on the choice of tile. This graph only shows the difference within treatment tanks, not comparing them to control tanks. Males had a slight tendency to more often choose the larger 10 cm x 10 cm tiles in higher disturbances.

**Table 2.** The impact of waves on the nest choice of gobies that built nests. Nest choice was analysed using a binomial generalized linear model.

	Estimate	Std. Error	z-value	p-value
<b>Nest choice</b>				
Intercept	1.140	5.291	0.216	0.829
Wave disturbance	0.035	0.058	-0.547	0.585
Goby length	-0.150	4.840	-0.031	0.975
Fulton's condition factor	-0.150	4.840	-0.031	0.975

### 3.3 Impact of wave disturbance on nest entrance width

The impact of wave disturbance on the width of the nest entrances in both treatments and controls was analysed using an ANCOVA. There was a tendency towards there being wider nest entrances in higher disturbances compared to lower disturbances ( $F_{1,16} = 1.379$ ,  $p = 0.223$ , Table 3). There was no relationship between the length of the goby and the width of the entrance ( $F_{1,16} = 1.379$ ,  $p = 0.508$ , Table 3) but a tendency

towards gobies with lower Fulton's conditional indices having wider nest entrances ( $F_{1.16} = 1.379$ ,  $p = 0.205$ , Table 3).

**Table 3.** The impact of waves on nest entrance width was analysed using an ANCOVA linear model. *Std. error* indicates the standard error and *t-value* indicates the t-statistic.

	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
<b>Nest entrance width</b>				
Intercept	64.560	27.249	2.367	0.031*
Wave disturbance	0.498	0.394	1.267	0.223
Goby length	-0.182	0.269	-0.677	0.508
Fulton's condition factor	-34.012	25.710	-1.323	0.205

### 3.4 Seasonal effects on nesting behaviour

A binomial generalised linear model analysis showed there was a correlation between the time of season a replicate was run and nest building, with gobies being less likely to build nests later in the season ( $df = 46$ ,  $p = 0.055$ ). Time of season had no effect on the choice of a smaller or larger tile ( $df = 21$ ,  $p = 0.696$ ) with any tendency towards gobies in experiments run later in the season being more likely to choose the smaller 6 cm x 6 cm. A linear regression showed that entrance size width was not impacted by time of season ( $F_{1.18} = 0.0384$ ,  $p = 0.847$ ).

**Table 4.** The impact of seasonal effect on nesting behaviour and nest choice was analysed using a binomial generalised linear model. The impact of seasonal effect on nest entrance width was analysed using a one-way ANOVA.

	<b>Estimate</b>	<b>Std. Error</b>	<b>z-value</b>	<b>p-value</b>
<b>Nesting behaviour</b>				
Intercept	1.035	0.653	1.584	0.113
Seasonal effect	-0.173	0.090	-1.917	0.055
<b>Nest choice</b>				
Intercept	0.171	0.780	0.220	0.826
Seasonal effect	-0.037	0.121	-0.391	0.696
	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
<b>Nest entrance width</b>				
Intercept	35.439	4.866	7.283	9.09e-07***
Seasonal effect	-0.138	0.705	-0.196	0.847

#### 4. Discussion

Although there was no effect of wave velocity on nesting behaviour, male sand gobies did show more tendency to build a nest in treatment tanks with lower wave disturbances. However, the gobies in higher disturbances were more likely to choose to build a nest under the larger 10 cm x 10 cm tile. The former of these results is in accordance with my prediction that males will build fewer nests in higher disturbances. I earlier postulated that this might be because the cost of nesting behaviour might be too high in a higher disturbance. There was no difference between whether a nest was built in controls versus disturbances and this trend was only found between the different wave velocities.

Studies on sticklebacks showed that males in poor condition had a smaller chance of building a nest in flowing water in comparison to a higher chance in still water (Rushbrook et al. 2010). Sticklebacks also showed less nesting behaviour when it was energetically too costly, such as when there was less food or when they were infested by parasites (Rushbrook et al. 2010). Whilst body condition, as measured by Fulton's condition factor, did not have an effect on nest building, there was a tendency towards males with lower body conditions being less likely to build a nest in these experiments. This may be for a myriad of reasons. Nest guarding is stressful, and the high amount of energy used can negatively impact the body condition by causing both weight loss and suppressed growth (Lindström 1992). Studies on the plainfin midshipman have shown that the constraint of parental care in the form of nesting behaviour may lower their body condition (Haupt et al. 2020). It may be that males with lower body conditions were less likely willing to build a nest in the experiment conditions and might not have built a nest in nature either. This tendency, whilst existing in all experiments, was more pronounced in tanks with wave disturbances and it can be that these trends were more pronounced when there was an added stress factor.

There are multiple ways to measure body condition in fish and Fulton's condition factor, whilst frequently used, is only one metric. A study on the bluegill sunfish found that Fulton's condition factor is more helpful in predicting lipid density as well as parasite density (Neff and Cargnelli 2004). It was also found that this condition factor is in general a good indicator of energy state and individual quality during breeding seasons (Neff and Cargnelli 2004). It is therefore likely to be a good

indicator for body condition of sand gobies in these experiments, but it is also important to remember that other factors can impact the condition of the males that may not be as visible or measurable as bodily reserves. More focus on body condition's impact on nesting behaviour would be interesting in a future study.

I also hypothesised that males would choose smaller tiles in higher disturbances to save on the cost of a larger nest requiring more maintenance in a higher wave velocity. This cost would not only include the building of the nest, but also the upkeep; fanning and defence have high costs to males (Lindström 1992). Larger nests are usually more prone to nest takeover from larger conspecifics as they are considered a more valuable resource (Lindström 1992). I earlier proposed that this would be to save energy, as the cost of building and keeping a larger nest is higher. Kvarnemo (1995) found that larger males would build nests under larger substrates and smaller at smaller sites, as the cost of maintenance of a too large nest in comparison to the size of the male is higher than the benefit. The results from this project, whilst again not statistically significant, showed a tendency that went against my hypothesis; gobies in higher wave velocities often chose larger tiles. There could be multiple reasons for this, including that larger nest sites, and therewith larger nests, might be more stable than smaller nests. Three-spined sticklebacks have been shown to use more spiggin (an adhesive-like glycoprotein used to reinforce the nest) when building their nests in flowing water than in still water (Rushbrook et al. 2010). This could possibly be to stabilise the nest more, to the extent that sticklebacks reared in these flowing waters have higher levels of spiggin gene expression compared to sticklebacks reared in still water (Seear et al. 2014). The amount of sand that was used to cover the nest was not checked in this project, which could have revealed if the males built more stable nests in higher wave disturbances. Another possibility is that a larger nest site is better for future offspring. Previous studies have found that males with larger nests have a higher hatching success rate as they have larger clutches (Forsgren 1997). The higher success rate can be due to an increase in parental investment (Forsgren 1997) and, especially in sand gobies, this can be seen by a lowered clutch cannibalism in nests with larger egg areas (Forsgren et al. 1996) and by a higher mating success at larger nests that may result in more offspring (Lehtonen and Lindström 2004, Lindström et al. 2006). One could postulate that due to the higher success rate in larger nests it is more beneficial for males to build a nest at a larger site in higher wave disturbances, although the cost itself is higher. In this way, only males that are capable of building

a larger nest in higher wave velocities will build a nest, as a smaller nest is not beneficial enough. Whilst it might be possible that the body condition of a male might affect its capability to build a nest in a higher disturbance, the body condition of male gobies did not affect the choice of nest site in these experiments. Gobies with lower body conditions are more likely to lose out on competition for nesting sites, causing lower reproductive success as females prefer males with larger nest sites (Barber 2002, Lindström 1988). As there was no competition for nesting site in the wave tanks, gobies of all body conditions had the possibility to choose either the larger or smaller nest site.

I found a trend that nest entrances were wider in higher disturbances, possibly due to the fact that the chosen nest sites were also larger, but also in the smaller nest sites that were chosen. Nest entrances usually vary in size, and changes are often made due to changes in the environmental conditions (Lehtonen et al. 2016). Larger nest entrances enable more ventilation, whilst smaller entrances are better for protecting eggs from predators (Lehtonen et al. 2016) and are, along with nest elaboration, positively correlated with mating success (Svensson and Kvarnemo 2005). It could be that this higher level of ventilation might enable the males to spend less time fanning, which is a drain of their energy even in non-disturbed environments (Kvarnemo 1995). This, however, is pure speculation and further research needs to be done to see if this is the case.

The results from this project did not always coincide with previous studies. My results showed that only 45.5% of gobies in controls chose the larger 10 cm x 10 cm tiles as nest sites. Some previous studies have shown that males in non-threatening environments tend to choose larger nesting sites, independent of the size of the male (Lindström 1988, Flink and Svensson 2015), whilst other contradictory studies have shown that this choice of nesting site is reliant on the size of the male (Kvarnemo 1995). My results also showed a significant trend that smaller males were more likely to build a nest in general, both in controls and in disturbances, whilst it has previously been thought that larger males were more likely to build a nest. Nest sites play an important part of male-male competition amongst sand gobies in nature (Magnhagen and Kvarnemo 1989) and larger males are often more successful in the competition for high-quality nest sites (Lindström 1992), although the success in male-male competition is irrelevant to female choice (Forsgren 1997). However, Kvarnemo et al. (1995) found in an aquarium study that this trend was mostly visible in male-biased

treatments and that there was no difference between the size of gobies that did and did not build nests in a female-biased treatment. The males in this experiment were alone in their tanks and there was, therefore, no competition for nesting sites, meaning that the smaller gobies that possibly would not otherwise have had a nesting site due to competition from larger gobies might have been able to build nests during the experiments.

Some of these differences from previous studies could be due to the wave tanks being a more stressful environment for the males in comparison to other aquaria. The experimental areas of the wave tanks are only 50 cm x 50 cm and the walls surrounding are metal. Previous studies on animals in wave tanks are few. A study on the effect of wave disturbances on predator-prey interactions showed that both the predator and prey changed their swimming pattern in the disturbed tanks in comparison to the controls (Gabel et al. 2011). That study, however, was performed in tanks with glass walls in comparison to the metal walls of the tanks in this project, as well as with a larger test section.

Upon further analysis, there was also a significant trend that the males were more likely to build a nest in earlier replicates. The experiments were run between the 2<sup>nd</sup> and 25<sup>th</sup> of June 2020. Sand gobies in the Baltic Sea have a reproductive season that usually stems between late May and August, meaning that these experiments were run through the earlier part of the season. This starting time was later than ideal, as I had hoped to begin the experiments in May and the late starting time may have affected the results of the project. Future experiments studying this possible correlation could be interesting.

For similar projects in the future, it could be interesting to have a larger range of wave velocities. I could not create a larger range of disturbances in these experiments, as I had to limit the pressure to 2 bars to maintain even waves for 23 hours that a replicate lasted. This experiment duration was chosen as it has been commonly used in other sand goby aquaria experiments. However, it would be interesting to see if these tendencies of less nest building and higher likeliness to choose a larger nest would become significant in more variable disturbance variations. Future studies should also include measurements of nest elaboration by measuring the amount of sand used to cover the tiles. This could probably have been included in this project as well.



Nesting behaviour in fish is easily affected by external forces. Exposure to endocrine disrupting chemicals has been shown to delay nest building in sand gobies (Saaristo et al. 2010). The nesting behaviour of many fish species decreases in polluted areas (Jones and Reynolds 1997) and naked goby males have been shown to abandon their nests when episodic hypoxia reaches lethal levels (Breitburg et al. 1997). Climate change has been shown to have affected the nesting behaviour of various animals, including some species of fish (Mainwaring et al. 2017) and it is thought that these nesting animals should adapt to the changing climate conditions. Simultaneously, there are also other factors that affect the breeding habitats of the littoral sand goby. Drifting algae can cover parts of the bottom and can cause oxygen deficiency as well as other stressful conditions (Norkko et al. 2000). The impact of a changing wave velocity is one more stress factor that male sand gobies may experience in the future.

I did not find that wave velocities between 8 cm/s and 18 cm/s had a significant impact on the nesting behaviour of sand goby males, but there were tendencies that showed that wave disturbance may lead to changes in nesting behaviour at even higher velocities.

## **5. Acknowledgements**

I would like to thank my supervisors Kai Lindstöm and Christoffer Boström for their support and advice during this project. I would also like to thank Lukas Meysick for his help with the wave tanks and Johanna Yliportimo for her help in the field. Finally, I would like to thank Societas pro Fauna et Flora Fennica for the scholarship that made this thesis possible.

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