# The noise of the hunt: Effects of noise on predator-prey relationships in a marine ecosystem

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## Citation

Kok, A. C. M. (2020, April 15). *The noise of the hunt: Effects of noise on predator-prey relationships in a marine ecosystem*. Retrieved from https://hdl.handle.net/1887/138192

**Author**: Kok, A.C.M.
**Title**: The noise of the hunt: Effects of noise on predator-prey relationships in a marine ecosystem
**Issue Date**: 2020-04-15

# The noise of the hunt

## Effects of noise on predator-prey relationships in a marine ecosystem

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The noise of the hunt:
Effects of noise on predators and prey in a marine ecosystem

PhD thesis, Leiden University, the Netherlands

Printed by Ridderprint, Ridderkerk
Cover design and lay-out by Annebelle Kok

# The noise of the hunt
## Effects of noise on predator-prey relationships in a marine ecosystem

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 15 april 2020
klokke 16:15 uur

door

Annebelle Cathelijne Marieke Kok
geboren te Leiden, Nederland
in 1989

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*I must go down to the seas again, for the call of the running tide*
*It's a wild call and a clear call that may not be denied*

(*Sea Fever*, by John Masefield)

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# 1

# General introduction

### Pollution in the marine environment

Animals living in today's marine environment face many threats. Many fish populations are in danger of collapse due to overfishing (Madin et al. 2016), while the various effects of climate change have the potential to affect all trophic levels in the ocean (Yao and Somero 2014). Oxygen levels in the oceans have decreased, leading to an expansion of oxygen minimum zones that can affect marine organisms and change predator-prey interactions (Breitburg et al. 2018). Another prominent threat is that of pollution. The accumulation of micro-plastics in marine waters is a familiar example (e.g. Zhang et al. 2019), but other forms of pollution do not leave any physical trace. One of those forms of pollution is sound pollution.

Awareness of sound pollution has emerged over the last decades. Since the invention of motorized ships, marine ambient noise levels have started increasing (Andrew et al. 2002) and have continued to rise as human activities in and above water have spread (Frisk 2012). Sound propagates well in water, so anthropogenic sound sources can affect ambient noise levels of large areas. A moored hydrophone at Ascension Island (equatorial Atlantic Ocean), picked up seismic air gun sounds year-round, possibly from seismic surveys thousands of kilometres away (Haver et al. 2017). In the Dutch part of the North Sea, anthropogenic sources constitute a large proportion of the sea's ambient noise levels (Sertlek et al. 2019). Although the increase in ambient noise levels has slowed down and even stopped in some areas – due to quieting of ships and little increase in ship movements  (Andrew et al. 2011; Miksis-Olds and Nichols 2016) – ambient noise levels are still substantially higher than pre-industrial noise levels.

### Effects of sound on marine life

These increasing levels of ambient noise affect marine animals (Slabbekoorn et al. 2010; Radford et al. 2014; Kunc et al. 2016). Direct detrimental effects include physiological changes, such as temporary or permanent hearing threshold shifts, to even mortality close to the sound source (Popper 2012). In addition, many animals are potentially affected in their behaviour, as effects of sound on behaviour may already occur at slight increases in ambient noise level (e.g. from 100 to 120 dB re 1 μPa, de Jong et al. 2018). Anthropogenic sound has been shown to affect a range of behaviours: from early-life events such as reef-settlement in larval reef fish (Simpson et al. 2016) to fitness-altering strategies such as mate choice in gobies, thereby also indirectly affecting the next generation (de Jong et al. 2018). The effects of noise span all trophic levels. Cetaceans, many fishes and even invertebrates have shown changes in behaviour in response to noise exposure (Sebastianutto et al. 2011; Blair et al. 2016; Hubert et al. 2018). Since all trophic levels can be affected, it is likely that marine ecosystems will be affected by noise exposure.

Sound characteristics such as level, frequency and amplitude all influence the potential impact of sound pollution. The frequency of the sound influences whether an animal can detect the sound and can potentially be affected by it. Many of the sounds produced by human activities are low in frequency, which overlaps with the hearing range of most fishes and invertebrates (Slabbekoorn et al. 2010). Frequency and sound level can also interact, as the frequency at which the greatest hearing threshold shift occurred for a harbour porpoise (*Phocoena phocoena*) changed with increasing exposure sound level of a sound that did not change in frequency (Kastelein et al. 2014). Furthermore, temporal structure of the sound influences the behavioural response. Impulsive sounds generate different response intensities than continuous sounds (Neo et al. 2014; Nichols et al. 2015; Shafiei Sabet et al. 2015; Radford et al. 2016; Kok et al. 2018). However, at the moment governmental policy makers only focus on trying to decrease the amplitude of ambient sound in the marine environment and disregard other acoustic parameters that might influence the effect on animals (PCEU 2008; Dekeling et al. 2016; Gomez et al. 2016).

The effects of sound on behaviour of marine animals have been studied with various methods. They have been studied with theoretical methods, for instance by creating acoustic propagation models and combining those with animal movement models (Aarts et al. 2016). Others have used indoor experiments, often by exposing fish in a tank to sound and recording their behavioural response, for instance in relation to foraging (Voellmy et al. 2014a; Shafiei Sabet et al. 2015). Increasingly, outdoor experiments have been used with controlled sound exposure (e.g. Miller et al. 2012; Neo et al. 2016) or behavioural observations of free-ranging animals that were exposed to uncontrolled anthropogenic sound events are conducted (e.g. McCarthy et al. 2011; Dyndo et al. 2015). All these types of study have their merits and pitfalls. Theoretical studies are useful for exploring effects of sound that would be logistically challenging to study otherwise, but the predictive value of the model relies on the accuracy of its assumptions (Starfield 1997). Indoor experiments are a great way to closely observe the behavioural response of the animal, but animals in captivity may behave differently than they would in free-ranging conditions (Garton et al. 2005). Outdoor experiments or observations record the natural behaviour of animals, but can be logistically challenging, often leading to incomplete datasets and small sample sizes, and in the case of observations, can only rarely show cause-effect relationships (Garton et al. 2005).

### *Impact of sound on predator-prey interactions*

Sound does not only affect single species, but is likely to also cause shifts in the ecosystem (Francis and Barber 2013). This can be caused by differences in sound impact between species. For example, species composition of a bird community in New Mexico changed in the presence of noise, from 32 species present at the quiet control site to 21 species at the noisy site (Francis et al. 2009). Similar results have been found for an avian community in Puerto Rico (Herrera-Montes and Aide 2011), although the anuran community in that area did not change its composition. In a marine environment, the call rate and call complexity of a fish community in the Mediterranean Sea was significantly lower at a noisy location than at a nearby pristine location (González Correa et al. 2019). The mechanisms underlying these changes in species composition can likely be related to species differences in behavioural impacts of noise. These could for instance be differences in avoidance of noisy areas between species, sound-related changes in competition strength of a species to competitor species at the same trophic level, or a sound-related shift in the balance between predator and prey species at multiple trophic levels.

The influence of sound on predator-prey interactions is a potentially important driver for ecosystem effects. Changes in predator-prey interactions may cascade through the food-web, thereby affecting not one but multiple species (Rao 2018). There are multiple aspects to the predator-prey interaction that can be influenced by sound (Fig. 1). The encounter rate between predators and prey depends on the spatial behaviour of both, which is influenced by habitat choice. Noisiness of the environment can influence this choice. Harbour porpoises, for instance, were temporarily excluded from certain parts of their habitat, due to pile-driving activities in that area (Carstensen et al. 2006; Tougaard et al. 2009; Dähne et al. 2013). When predators hunt prey, sound may directly influence the performance of the predator (Voellmy et al. 2014a; Shafiei Sabet et al. 2015) or the anti-predator behaviour of the prey (Voellmy et al. 2014b; Simpson et al. 2016). Many cetacean predators are highly social animals, while groups split up when foraging. Sound may decrease the communication range of group members (Janik 2000; David 2006; Miller 2006), making it more difficult to relocate the group at the end of the foraging bout. Finally, the changes in behaviour caused by sound may alter time-budgets for other behaviours (Isojunno et al. 2017), for instance such that prey that become more vigilant have less time for other behaviours, such as foraging (Voellmy et al. 2014a).

Despite this general knowledge, there are many gaps in understanding the effects of sound on predator-prey interactions. In cetaceans, effects of sound are difficult to study, because controlled indoor experiments are generally not possible. Controlled outdoor experiments have been increasing in number and have led to great advances in understanding the effects of sound on marine animal behaviour (Miller et al. 2012; Dunlop et al. 2016), but still are logistically challenging and costly. Furthermore, it becomes more and more evident that responses to sound differ between sound types, as well as between species. In fish, effects of noise on anti-predator behaviour fall into two categories: fish get distracted or oppositely, increase their vigilance. It is unclear how these processes relate to each other, let alone why one species gets distracted while the other does not (Rosa and Koper 2018). The largest prey taxon, invertebrates, has hardly been studied at all, even though this group is likely to be sensitive to low-frequency sound, which is the frequency range of most anthropogenic sound sources (Slabbekoorn et al. 2010; Morley et al. 2014).

## *Outline of the thesis*

This thesis specifically investigates the effects of noise on predator-prey relationships. Using a multidisciplinary approach, I investigated various stages in the process of predator-prey interactions that can be influenced by sound (Fig. 1). The thesis is subdivided in two sections. The first section investigates how noise affects foraging predators (Chapters 2 to 4), while the second section investigates how noise affects prey (Chapters 4 & 5). I studied two cetacean predator species: harbour porpoises (*Phocoena phocoena*) and long-finned pilot whales (*Globicephala melas*). As prey species, I studied

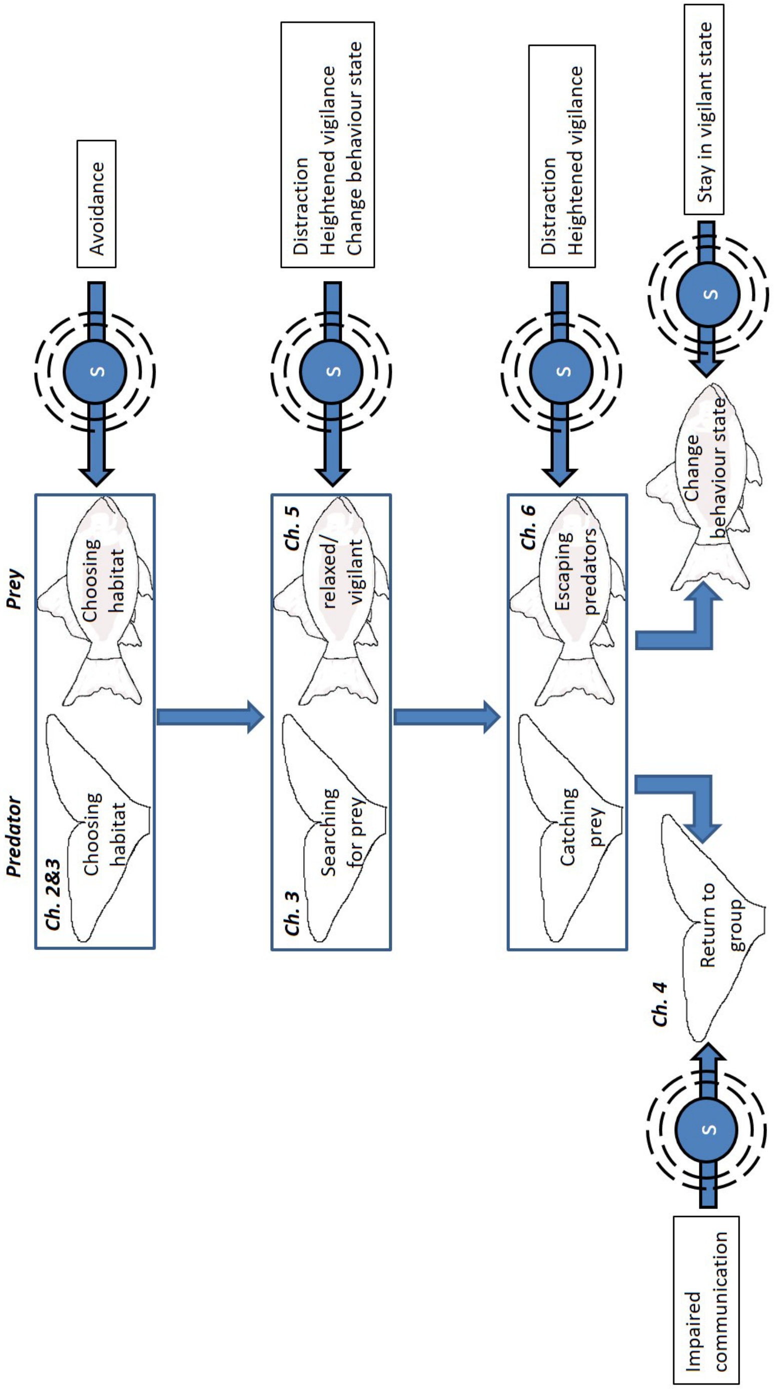

Figure 1: Schematic overview of the stages in predator-prey interactions in which anthropogenic noise can have an influence.

sand gobies (*Pomatoschistus minutus*) and pelagic fish of the North Sea. Because of the varying demands of each study, I combined indoor and outdoor experiments with behavioural observations and theoretical modelling, thereby benefitting from the specific advantages of each method. Combined, these studies aim to advance insight in the behavioural responses of predators and prey to anthropogenic noise.

## *How noise may affect a foraging predator*

When a predator starts foraging, it first has to locate a suitable habitat. Foraging habitat quality is regulated by many factors, such as the availability of prey and the risk of being predated. Anthropogenic noise can alter habitat choice by deterring animals from a particular area. Harbour porpoises have been observed to evade wind farm construction sites during pile driving events (Carstensen et al. 2006; Tougaard et al. 2009; Dähne et al. 2013). However, these were correlational studies that did not investigate whether the observed avoidance was a direct deterrent effect of the noise on the porpoises, or an indirect consequence of other noise-induced changes, such as prey leaving the area. In chapters 2 and 3, I investigated swimming behaviour of two captive harbour porpoises in control conditions (chapter 2) and under artificial noise exposure (chapter 3). Furthermore, I studied the influence of sound pressure level (SPL) and temporal sound structure on the probability of avoidance.

After finding a suitable habitat to forage, a predator has to search for a specific prey item. This process can be disturbed by sound in multiple ways. First, anthropogenic noise can cause a change in behavioural state, meaning the predator stops foraging and starts an alternative behaviour, such as travelling. This response type has been documented for several cetacean species (Miller et al. 2012; DeRuiter et al. 2013). Second, anthropogenic noise can cause distraction from prey searching, lowering performance levels. In chapter 3, I studied how noise affected prey searching in harbour porpoises that were trained to perform a prey search task by locating a dead fish in one of three possible cages. By varying temporal structure and sound level of the noise, I investigated the influence of these sound characteristics on the probability of distraction.

Finally, when a predator has finished foraging it will return to another behavioural state. Many odontocetes are deep-diving marine predators, which forage individually at depth but form stable social groups at the surface after foraging is over. In order to relocate their group at the surface, odontocetes are likely using acoustic communication. Increasing ambient noise levels might disrupt communication between separated individuals. In chapter 4 I tested the potential for pilot whale calls to be used for acoustic relocation when animals were vertically separated and investigated whether environmental variables such as ambient noise level at the receiver, as well as call characteristics, influenced communication potential.

## *How noise may affect prey*

The prey must be vigilant for a sudden encounter with their predator. This vigilance for predation has a trade-off with other behaviours, such as foraging, and can therefore be altered under the influence of noise in two ways. On the one hand, prey could be distracted by the noise, thereby becoming less vigilant (Chan et al. 2010). On the other hand, prey could perceive the sound as a risk to be predated and respond as if a predator was close (Frid and Dill 2002). In chapter 5, I investigated whether the behaviour of North Sea pelagic fauna changed during exposure to a seismic survey and pile driving. I recorded whether pelagic biomass showed vertical displacement during sound exposure and whether schooling organisms changed their cohesion, both behavioural traits that also change as part of anti-predator behaviour (Rieucau et al. 2014).

At the moment of a predator attack, prey will try to escape the predator. Again, prey can be distracted by the noise and react more slowly to the predator than they would otherwise. A direct consequence is an increase in mortality (Simpson et al. 2016), thereby favouring selection for individuals that show little distraction by noise, or are vigilant, vs. those that become distracted . If an exposed individual does not get eaten, an alternative consequence might be habituation to the noise exposure over time. Thus, in areas with long-term exposure to anthropogenic noise animals are likely to be overall less responsive, or more vigilant to the noise than in areas that have not been exposed to noise. I tested this hypothesis in chapter 6 with sand gobies living in areas with varying degrees of exposure to boat noise, which I exposed to a simulated predator under ambient or boat noise conditions.

All the effects on predators and prey together paint a complex picture of the effect of noise on the marine ecosystem. Chapter 7 provides a synthesis of the results of the previous chapters. I summarise the effects that I found on predators and on prey in this thesis and discuss how these results might be put into ecosystem context. Bridging all the knowledge gaps in the effects of noise on predators and prey is impossible with one thesis, so chapter 7 also suggests future directions for this line of research.

# *How noise affects foraging predators*

# Spatial behaviour, swimming speed and surfacing rate of two captive harbour porpoises in ambient sound control conditions

Annebelle C.M. Kok, J. Pamela Engelberts, Fleur Visser & Hans Slabbekoorn

*Reproduced with minor corrections from publication in Proceedings of Meetings on Acoustics, 2016, 27, 010037*

## Abstract

Foraging is made up of three steps: first, to search and encounter a suitable foraging patch; next prey has to be found, and finally the prey has to be caught and eaten. Behavioural responses to anthropogenic noise may lead to a disruption of vital activities within this process, such as area avoidance, or reduced abilities to locate or catch prey. Gaining insight into these behavioural effects starts with a thorough understanding of within- and between-individual variation in the baseline behaviour of experimental conditions. In this study, we analysed control trials for two captive harbour porpoises that were tested for spatial behaviour in a set-up for experimental sound exposure. Data from trials without any experimental sound exposure were used to investigate relationships between the response variable – time spent away from the preferred area – and two other behavioural metrics: swimming speed and surfacing rate. The results show that these metrics can be used as independent measures, the first being correlated to the response variable, the second uncorrelated. Combining the two makes a better and more complete judgment. Performing an adequate exploration of the variation in behaviour during control trials is important, as it will aid scientists in revealing and interpreting effects of disturbance in sound exposure trials.

## Introduction

Eating is important to growth, survival and reproduction for most animals and foraging is therefore a critical behaviour contributing to individual fitness. Foraging is a process that can be made up of three steps: exploring the home-range to select a suitable foraging area; finding prey and approaching specific food items; and actually catching prey, including handling and swallowing of the food (Kamil and Roitblat 1985). For carnivorous species, this involves hunting, an activity that can require quite a bit of effort, since it is in the best interest of the prey not to be caught (e.g. Aguilar de Soto et al., 2008). The efficiency with which an individual forages is directly correlated to the energy balance that will translate into fitness. Any disturbances that impede foraging can therefore have detrimental fitness consequences.

Anthropogenic noise is such a disturbance. On all three levels of the foraging process, evidence of a negative impact of noise has been found. Daubenton's bats (*Myotis daubentonii*), for instance, avoided noisy parts of an arena, even though food could be found at those sites (Luo et al. 2015). Similarly, deterrence from potential food patches where noise levels were temporarily elevated, such as wind farm construction sites, has been documented in the wild for harbour porpoises (*Phocoena phocoena*) (Carstensen et al. 2006; Dähne et al. 2013), although not in all cases (Scheidat et al. 2011; Thompson et al. 2013). Any causal relationship between anthropogenic noise and avoidance in harbour porpoises has not been investigated to date.

For finding and catching prey, results from fish behaviour studies have shown that anthropogenic noise can result in lower foraging performance, potentially due to attention shifts. Shafiei Sabet et al. (2015) tested the foraging performance of zebra fish (*Danio rerio*) under relatively quiet ambient and noisy conditions, and found that individuals delayed their initial acceleration and made more handling errors for noisy compared to ambient conditions. Sticklebacks (*Gasterosteus aculeatus*) showed similar responses when exposed to noisy conditions, making more mistakes while maintaining an equally high foraging effort as during ambient conditions (Voellmy et al. 2014a). Foraging performance in these species appears not to rely on the use of sound, either actively or passively, implying that distraction by the noisy environment is a more likely mechanism underlying the disturbance. However, not all fish species show the same response pattern. In contrast to sticklebacks, European minnows (*Phoxinus phoxinus*) in the same study ceased foraging altogether and were inactive for most of the elevated sound level treatments (Voellmy et al. 2014a). Qualitatively, this could be compared to an avoidance response. Overall, it seems that two mechanisms can yield negative effects of anthropogenic noise on foraging efficiency: stress-related avoidance (reduced opportunity) and attention-related performance drop (missed and failed opportunity).

In harbour porpoises, effects of anthropogenic noise on foraging efficiency could have direct fitness consequences. As small marine mammals, their surface-to-volume ratio is relatively high, which means that they need relatively large amounts of food to survive (Feldman and McMahon 1983). This has been corroborated recently by investigating click patterns and swimming behaviour in tagged wild harbour porpoises (Wisniewska et al. 2016). Individual harbour porpoises were found to consume up to 550 small prey items per day, with a 90% success rate per attack. The high prey number and low energetic content per prey suggest that these high feeding rates are necessary for survival of the porpoises. Even a small decrease in foraging efficiency could potentially have large fitness consequences.

Behavioural assessments of stress can be challenging, since the internal motivation of the animal is unknown. An animal that is very active when faced with an external stimulus could be stressed, but it could also be positively excited by the stimulus. In order to disentangle the two, physiological measurements of cortisol level are often taken alongside behavioural measurements. This way, an increased activity level was related to an increased stress level for spiny lobsters (*Palinurus elephas*) (Filiciotto et al. 2014). Spiny lobsters were exposed to either boat noise or a control condition. When exposed to the boat noise, they showed both increased cortisol levels and an increase in activity, indicating that heightened activity can be a sign of stress.

Respiration rate has been shown to be a good proxy for cortisol levels in both terrestrial (e.g. great tits, *Parus major*; Carere and van Oers, 2004) and aquatic animals (e.g. Nile tilapia, *Oreochromis niloticus*; Barreto and Volpato, 2011). In a case study with a harbour porpoise, an increased respiration rate was linked to sound exposure, suggesting a relationship between sound exposure and stress (Kastelein et al. 2015a). In the same study, an increase in swimming speed was reported in response to sound exposure, relative to the baseline. It would be possible, therefore, that respiration rate and swimming speed are positively correlated.

However, changes in respiration rate and general activity level are not necessarily directly related in odontocetes. In killer whales (*Orcinus orca*) respiration rate was better predicted by a model assuming varying $O_2$ uptake than a model assuming a fixed $O_2$ uptake (Roos et al. 2016). A consequence of this was that respiration rate was a poor predictor of metabolic rate for low swimming speed. Only when swimming speed was high, and most of the oxygen stores were depleted before the next respiration, did respiration rate become directly correlated to metabolic rate. In harbour porpoises, it remains to be seen if these two parameters are directly correlated or not.

In this light, we here introduce our work on foraging efficiency in two captive harbour porpoises and provide insight into the quantification of behavioural parameters. We performed a dual-tank experiment to investigate avoidance behaviour in response to sound, testing for stress-related avoidance. In order to be able to test for this mechanism we conducted a pilot study in which we investigated the natural variation in our response metric – time spent in the quiet pool – and related it to activity level and respiration rate during control trials. We hypothesize that time spent in the quiet pool will be positively correlated to activity, because if the chance of transiting between pools remains the same, a higher swimming speed will cause the time between transitions to decrease and therefore the time spent in the quiet pool to increase. At the same time, time spent in the quiet pool will not be related to respiration rate, since we expect no increase in stress level during the control trials – which could increase respiration rate – and no direct relationship between respiration rate and activity level. By comparing these behavioural variables, we determined which metric, or combination of metrics, would be a suitable proxy for stress in the exposure trials. The methods section covers both control and exposure trials, while the control trials were critical in this first step of the analyses. The overall results of the experiment are reported in chapter 3.

## Materials and methods

### *Experimental setup*

Two captive harbour porpoises, one male and one female, were exposed to a silent control or band-passed brown noise (400-2000 Hz) for 15 min. The sound could be either intermittent (50 ms pulse every 2 s) or continuous, and was presented at multiple SPLs. The animals were allowed to move freely between two connected pools, an indoor and an outdoor pool (Fig. 1). The outdoor pool was slightly larger than the indoor pool, 12x8 m vs. 8x7 m respectively. Sound was played in the outdoor pool, while the indoor pool stayed relatively quiet. For this study, only the control trials were analysed. All trials were recorded from above using cameras (outside: two cameras, one Go Pro Hero 3, one Conrad 750940; inside one Conrad 750940). In total, 21 control trials were conducted over a period of one month, between 10:30 am and 5:30 pm. Of these trials, 17 were analysed due to time constraints.

### *Analysis*

Spatial behaviour (time spent in either pool, number of transitions between pools) was measured per individual for every trial. A proxy for respiration rate was taken by counting the number of surfacing events per individual for every trial. Tracking software

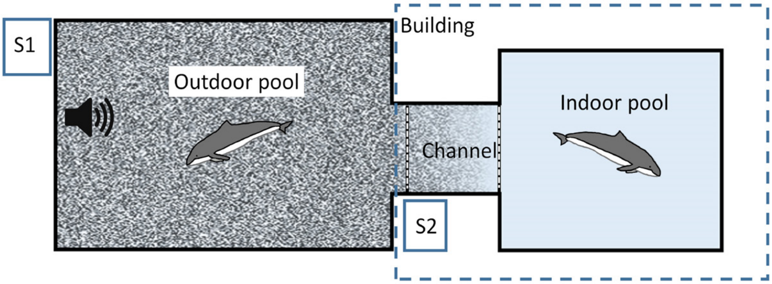

Figure 1: Schematic representation of the experimental setup. The harbour porpoises could move freely between the outdoor and indoor pool via the channel. Researchers conducting the experiments were out of view in cubicle S1 and S2. Black/white shaded area in top left pool indicates the area with elevated sound levels. Interrupted black and white lines to the left and right of the channel indicate gates that could close off the indoor from the outdoor pool. The interrupted blue line denotes the outer rim of the building in which the right-hand pool was situated.

(Kinovea 0.8.15) was used to track both individuals during the first two and last two minutes of the exposure period. This provided a distance in number of pixels moved, which was divided by the number of frames to calculate swimming speed. The swimming speed was then averaged over both sampling periods per trial. Due to the low quality of recordings of the indoor camera, swimming speed was only measured when individuals were in the outdoor pool.

Behaviour patterns related to time spent in the indoor pool were investigated by testing for correlations between measurement variables. As none of the variables were normally distributed, a Spearman rank correlation was performed for all comparisons. A Wilcoxon rank sum test was used to see if male and female samples could be grouped together, or had to be tested separately. Furthermore, time spent in either pool was tested for skewedness (taking into account the different sizes of the pools) using a one-sample Wilcoxon test, to investigate a preference for either pool. Only data from control trials were used, to prevent any influence of the sound exposure. All statistical analyses were performed using RStudio (www.r-project.org).

### *Ethical statement*

All experiments were performed in accordance with National and European legislation on proceedings concerning animal testing. Animals were always able to avoid the sound and trials would be ceased if strong adverse effects were seen (the latter was, however, never the case).

Table 1: Time inside, swim speed and transitions between the outdoor and the indoor pool correlated with each other, while surfacing rate was not correlated to any of the other variables. Spearman rank correlations, N = 17 for all tests.

| | **Time inside** | | **Surfacing rate** | | **Swim speed** | | **Transitions** | |
|---|---|---|---|---|---|---|---|---|
| | **Female** | **Male** | **Female** | **Male** | **Female** | **Male** | **Female** | **Male** |
| **Time inside** | x | x | n.s. | n.s. | $p < 0.0001$<br>$\rho = 0.8$ | n.s. | $p < 0.005$<br>$\rho = 0.7$ | $p < 0.0001$<br>$\rho = 0.9$ |
| **Surfacing rate** | | | x | x | n.s. | n.s. | n.s. | n.s. |
| **Swim speed** | | | | | x | x | $p < 0.05$<br>$\rho = 0.6$ | n.s. |
| **Transitions** | | | | | | | x | x |

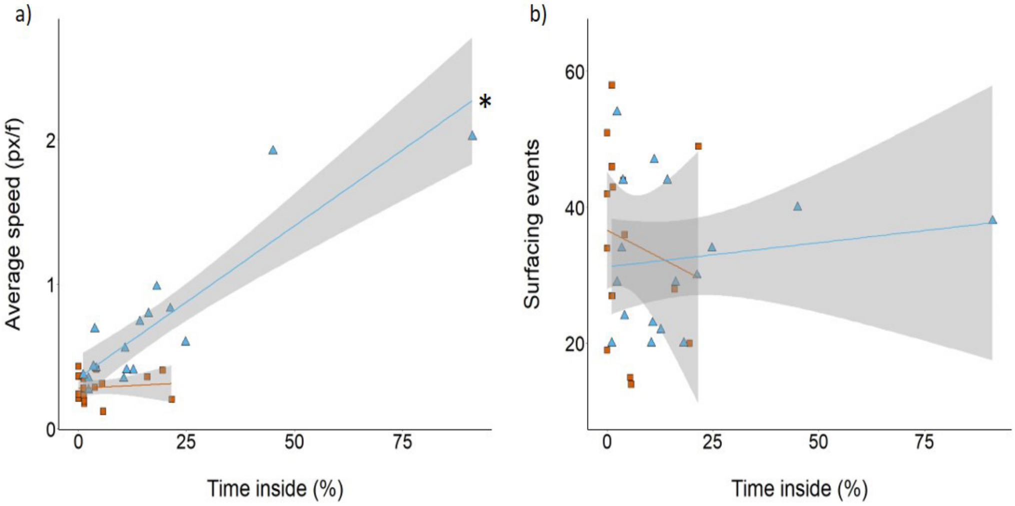

Figure 2: The percentage of time the female (blue lines and triangles) spent indoor was significantly correlated to a) the average speed (in pixels per frame) but not correlated to b) the number of surfacing events. Time inside of the male (orange lines and squares) was not correlated to either speed or surfacing events. The asterisk indicates a significant trend line.

## Results

For all variables except surfacing events, male and female samples were significantly different and could therefore not be tested in a single group. For both individuals, the percentage of time spent in the indoor pool was significantly lower than chance (Wilcoxon signed rank test, female: V = 22, p<0.005; male: V = 0, p<0.0001, N = 21). The percentage of time spent in the indoor pool was correlated to average swimming speed for the female, but not the male (Spearman rank correlation, Table 1, Fig. 2a). Visual inspection of the plot indicated two outliers for the female. The correlation was rerun without these outliers, but the outcome was similar. Consequently, we decided to keep the outliers in the data set.

The percentage of time spent in the indoor pool and the number of surfacing events were not correlated (Table 1, Fig. 2b). Neither was the number of surfacing events correlated to swimming speed or the number of transitions between pools (Table 1). However, the number of transitions between pools was correlated to the percentage of time spent in the indoor pool and for the female to the average swimming speed (Table 1).

## Discussion

The purpose of this study was to find metrics of harbour porpoise behaviour that would be a suitable proxy for stress. Since stress on a behavioural level is often difficult to disentangle from other behavioural modes, we investigated the natural variation of our response variable, time spent in the quiet pool, during control trials and compared it to activity and surfacing rate. These last two are both behavioural parameters that have been related to stress in the past. Time spent in the quiet pool was positively correlated to swimming speed, but only for the female. Surfacing rate was not correlated to time spent in the quiet pool for either individual.

On the whole, both animals spent more time in the outdoor than the indoor pool, suggesting a preference for the outdoor pool. Therefore, for this set-up, the time spent in the indoor pool can be seen as time spent away from the preferred area. This has important implications for our exposure study, for even if the change from outdoor to indoor pool would be caused by a change in activity rather than stress, the consequence would still be that the animals spend more time outside of the area they prefer under ambient conditions.

As hypothesized, we found a positive correlation between time spent in the indoor pool and swimming speed. Interestingly, however, this correlation was only present for the female, not for the male. Since the study was conducted with two animals, it is not possible to pinpoint the exact nature behind this difference. It could be due to a difference in sex, personality, previous experience or age, as these factors all differed between the two individuals. Furthermore, the small sample size of this study prevents us from making any generalized predictions about correlations between time spent in an area and swimming speed. What it does show, is that individual differences need to be taken into account when conducting these types of studies.

Individual differences in response to noise have been documented for other species as well. For example, nesting female cichlids (*Neolamprologus pulcher*) were less likely to defend against an egg predator under noisy conditions, whereas males showed no difference between elevated noise and control conditions (Bruintjes and Radford 2013). In another study, eels startled less from a model-predator under noisy conditions compared to control, but only if their body condition was poor (Purser et al. 2016). In both cases, differences in response pattern could be attributed to individual-specific parameters, namely sex and body condition. Knowing which factors contribute to intraspecific variation aids in predicting population-level effects.

The positive correlation between time spent in the indoor pool and activity level can indicate two things. First, it could be that there was a certain likelihood for the female to transit to the other pool when passing the opening. An individual with a higher swimming speed would pass the opening more often during the same period, consequently spending more time in the indoor pool. Second, it could indicate that the female changed her preference to the indoor pool when active. Since the number of transitions was also positively correlated to both time spent in the indoor pool and swimming speed, it is probable that the first explanation holds.

Avoidance and swimming speed have been investigated together for other sound exposure studies. Zebra fish that were exposed to noise in a dual-tank set-up, increased their swimming speed, but did not spend more time in the quiet tank (Neo et al. 2015a). In contrast, a study on the effect of sound and light by Shafiei Sabet et al. (2016) found the opposite: when exposed to sound, zebra fish responded by freezing, instead of speeding up. Furthermore, these fish did avoid the sound source, albeit only the area right in front of the speaker. These two examples show that the relationship between avoidance and activity can vary even within a species, stressing the need for a thorough examination of the baseline behaviour of the study organisms.

In line with our predictions, surfacing rate was uncorrelated to the percentage of time animals spent in the indoor pool. As an increase in respiration rate has been linked to increased stress hormone levels in other species, it is safe to conclude that time spent in the indoor pool is not linked to stress for this data set. Since the data were collected during control trials, one would not expect a change in stress level. However, if a positive correlation between surfacing events and time in the indoor pool arises during the exposure trials, this will indicate a change in function of the indoor pool from just 'extra space' to a preferred area in times of stress.

The lack of relationship between surfacing events and swimming speed for both animals is in line with similar findings for killer whales (Roos et al. 2016). The oxygen uptake for each surfacing event likely varied, due to a variable store of oxygen in the body. Since the data for this study were collected during control conditions, the animals were likely to only show low to average activity levels. Thus, it is unlikely that their oxygen stores were depleted for every surfacing event. The findings of Kastelein et al. (2015a) – an increase in swimming speed combined with an increase in respiration rate in response to noise – are therefore most likely caused by stress acting independently on both response types.

### *Conclusion*

The results of this study show that within- and between-individual variation can be present in metrics normally used for measuring avoidance and stress in sound exposure studies. Surfacing rate and swimming speed, metrics that are often correlated in other species, can be considered as two independent behavioural measures for the two harbour porpoises used in this study. Only by combining the two, however, is it possible to get a suitable behavioural proxy for stress, which is necessary to judge the impact of sound exposure. Performing an adequate analysis of the variation in behaviour during control trials is important, as it will aid scientists in elucidating effects of disturbance in exposure trials.

## Acknowledgments

We are indebted to Manon Horvers, Kysha Kraaijvanger, Rosanna Toma, Lean Helder-Hoek, Shirley van de Voorde and Ron Kastelein for helping us with the data collection at SEAMARCO, Erwin Jansen of TNO for performing the calibration measurements and the department of Behavioural Biology at Leiden University of providing feedback on the experimental set-up. Funding was provided by LUF, Gratama Stichting and the Dutch Ministry of Infrastructure and the Environment.

# Spatial avoidance to experimental increase of intermittent and continuous sound in two captive harbour porpoises

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*Published in Environmental Pollution, 2018, 233, 1024:1036*

## Abstract

The continuing rise in underwater sound levels in the oceans leads to disturbance of marine life. It is thought that one of the main impacts of sound exposure is the alteration of foraging behaviour of marine species, for example by deterring animals from a prey location, or by distracting them while they are trying to catch prey. So far, only limited knowledge is available on both mechanisms in the same species. The harbour porpoise (*Phocoena phocoena*) is a relatively small marine mammal that could quickly suffer fitness consequences from a reduction of foraging success. To investigate effects of anthropogenic sound on their foraging efficiency, we tested whether experimentally elevated sound levels would deter two captive harbour porpoises from a noisy pool into a quiet pool (Experiment 1) and reduce their prey-search performance, measured as prey search time in the noisy pool (Experiment 2). Furthermore, we tested the influence of the temporal structure and amplitude of the sound on the avoidance response of both animals. Both individuals avoided the pool with elevated sound levels, but they did not show a change in search time for prey when trying to find a fish hidden in one of three cages. The combination of temporal structure and SPL caused variable patterns. When the sound was intermittent, increased SPL caused increased avoidance times. When the sound was continuous, avoidance was equal for all SPLs above a threshold of 100 dB re 1 μPa. Hence, we found no evidence for an effect of sound exposure on search efficiency, but sounds of different temporal patterns did cause spatial avoidance with distinct dose-response patterns.

## Introduction

In the last decades, anthropogenic sound levels in the oceans have risen (Andrew et al. 2002). At the same time, an increasing body of research is showing a variety of disturbance effects from man-made sounds on marine life (Richardson and Würsig 1997; Popper and Hastings 2009; Slabbekoorn et al. 2010). Effects range from physiological effects such as increased stress levels (Debusschere et al. 2016; Filiciotto et al. 2016) to behavioural effects such as startle and freezing responses (Kastelein et al. 2008a; Shafiei Sabet et al. 2016), avoidance (Buck and Tyack 2000; Miller et al. 2012), reduced signal detection efficiency due to masking (Radford et al. 2014; Erbe et al. 2016) and decreased effectiveness of anti-predator responses (Simpson et al. 2016). Effects have been reported across all trophic levels and most marine taxa, including cetaceans, fish, and crustaceans (Popper and Hastings 2009; Filiciotto et al. 2016; Southall et al. 2016).

A main potential consequence of anthropogenic sound of current concern is a reduction in the time and space available for foraging, due to avoidance of suitable areas (Siemers and Schaub 2011; Wisniewska et al. 2016). For acoustic predators, noisy conditions may mask prey sounds, thereby impeding foraging performance. This has been demonstrated for several terrestrial animals. Greater mouse-eared bats (*Myotis myotis*) more often avoided a noisy side of an experimental foraging arena and preferred the quieter side when sound was played back (Schaub et al. 2008). When the frequency spectrum of the sound overlapped sound spectra of their rustling prey, avoidance was further increased. Furthermore, noisy conditions may lead to spatial avoidance of suitable foraging habitat, even if sound does not mask prey sounds. Daubeton's bats (*Myotis daubetonii*), for example, avoided noisy food patches, even when the sound did not spectrally overlap with click echoes from the prey (Luo et al. 2015).

For species that do not use sound to locate their prey, reduced foraging performance may arise from sound affecting the animal's stimulus-processing capacities. Zebrafish (*Danio rerio*), for example, feeding on water fleas delayed their initial acceleration towards prey and made more prey-handling errors in the presence of intermittent sound (Shafiei Sabet et al. 2015). Three-spined sticklebacks (*Gasterosteus aculeatus*) also made more prey-handling errors, and in addition were less able to discriminate between food items and non-edible particles (Purser and Radford 2011). These effects seemed to be caused by a cross-modal effect on sensory channels involved in prey hunting and processing (Halfwerk and Slabbekoorn 2015) or by a general drop in performance due to an inherent diffusing effect on attention (Chan et al. 2010).

Anthropogenic sound can simultaneously cause avoidance and distraction in a single species (Schaub et al. 2008). To separate the two mechanisms, Luo et al. (2015) designed a foraging task in which bats could either avoid sound, or maintain foraging effort and possibly increase their error rate through distraction. They did not, however, investigate what would happen if the bats were forced to forage in sound that they would otherwise avoid, a likely situation if available food is scarce and located in noisy areas. On the one hand, suppressing aversion to the sound might increase the impact of sound on attention, thereby causing a larger disturbance. On the other hand, situations may occur in which the sound is not aversive, but might still create an attention shift (Neo et al. 2015a; Shafiei Sabet et al. 2015). Indeed, ambient sounds can be both attractant and deterrent depending on their level and temporal pattern (Nelson and Johnson 1972). How such effects of sound combine and result in certain degrees of avoidance and distraction may vary per sound and species, and requires much more research.

The temporal structure and amplitude of sound can influence the nature and threshold of behavioural responses in animals. Several fish species, for example, respond differently to the same sound when it is pulsing (intermittent sound) than when it is continuous (Neo et al. 2014; Nichols et al. 2015; Shafiei Sabet et al. 2015). At equal exposure levels, intermittent – but not continuous - sound caused fish: to increase cohesion and dive deeper (Neo et al. 2014); to display a stress response (Nichols et al. 2015); or to reduce their capability to discern food particles (Shafiei Sabet et al. 2015). It is furthermore generally accepted that the probability of a response rises with increasing sound levels, yielding typical dose-response curves (see Hawkins et al., 2014a; Miller et al., 2014). Since temporal variety is common in anthropogenic sounds of marine soundscapes, it is important to investigate their effects on response thresholds in more animal species.

Harbour porpoises (*Phocoena phocoena*) are acoustic predators that use echolocation clicks to locate and capture prey (Kastelein et al. 1995b; DeRuiter et al. 2009; Wisniewska et al. 2016). They occur in areas with frequent noisy human activities, such as the Dutch North Sea (Scheidat et al. 2012; Sertlek 2016). Field studies at wind farm construction sites suggest that the combination of pile driving, construction and vessel sounds may induce spatial deterrence (Carstensen et al. 2006; Tougaard et al. 2009; Dähne et al. 2013), although porpoises sometimes seem to persist in the noisy area (Scheidat et al. 2011). Furthermore, distraction by sound can cause harbour porpoise bycatch in gill nets (Kastelein et al. 1995a; Nielsen et al. 2012; Wright et al. 2013), while general incapability of net detection by porpoises is unlikely, as they have been shown to be able to avoid gill nets at long ranges (Nielsen et al. 2012). In an experimental, captive study, a porpoise exposed to pile driving sound increased its respiration rate and started breaching (Kastelein et al. 2015a). Harbour porpoises are warm-blooded marine predators with a relatively high surface-to-volume ratio. Consequently, even a small decrease in foraging opportunity or efficiency due to spatial avoidance and decreased hunting performance due to sound exposure may have fitness effects on individuals (Kastelein et al. 1997; Lockyer et al. 2003; Wisniewska et al. 2016).

To investigate effects of anthropogenic sound on spatial behaviour and prey-search efficiency, we exposed two captive harbour porpoises to increasing levels of low-frequency, broadband, artificial sound. First, we investigated if an increase in sound levels could deter porpoises from an area, by creating a noisy and a quiet pool in a connected pool setup. Second, we tested if elevated sound levels could impede performance in a food searching task where the porpoises had to find a fish hidden in one of three cages. In Experiment 1, we used a range of sound levels, from which we selected a low and high level for Experiment 2. In both experiments, we quantified behaviour during exposure to either intermittent or continuous sound to compare to behaviour during ambient control conditions. We hypothesized that 1) elevated sound levels can induce spatial avoidance and a performance drop and that 2) sound impact will be higher for intermittent and louder sounds.

## Materials and methods

### *Study subjects*

Experiments were conducted on two captive harbour porpoises (*Phocoena phocoena*), one female (age 5.5 years, average weight 43.5 kg) and one male (age 2.5 years, average weight 33 kg). Both animals had stranded earlier in life and were rehabilitated by SOS Dolfijn, the Netherlands before being relocated to the research facility of SEAMARCO (the Netherlands). Each individual was given a set weight of herring and sprat divided over five feeding times per day, based on weight, motivation and predicted ambient temperatures (female: ~2500 g; male: ~1600 g). The hearing of the animals had been tested prior to the start of the first experiment. Both animals had normal hearing curves, which did not differ significantly from each other (Kastelein et al. 2017). The animals received enrichment in the form of balls and buoys. These were removed before the start of an experiment and replaced after experiments for that day had finished. All experiments were carried out in accordance with national and European legislation on animal testing. Both animals were always able to avoid exposure to the sound by swimming to a quiet pool. In Experiment 2, animals could refuse to take part in a trial, in which case it was suspended till a later time. During the experiments, the behaviour of the animals was constantly monitored and a trial was to be ceased if a strong aversive response was observed (this was never the case).

### *Research area and general procedures*

The animals were housed together in two connected pools, one indoor (8x7 m, 2 m deep) and one outdoor (12x8 m, 2 m deep; Fig. 1; see Kastelein et al., 2012 for a detailed description). Both pools were connected by a channel (4x3 m, 1.4 m deep) that could be closed off. The water in the pools was refreshed at night by water from the Oosterschelde. Before entering the pool, half of the water was pumped through a bio-filter system. The pumps were switched off 15 minutes before experiments started, to avoid confounding effects of sounds produced by the pumps. The average water temperature was 6-12 °C, similar to the water temperature of the Oosterschelde during the experimental period, with a salinity around 1800 mg Cl/L. Due to the remote location of the facility, ambient sound levels in and around the pool were relatively low and not affected by car or boat traffic.

Experiment 1 was designed to test if artificial brown noise could induce avoidance behaviour in captive harbour porpoises and if the sound pressure level (SPL) or temporal structure of the sound would affect this behaviour. Using a dual-pool setup, sound was played in a ‘noisy’ pool, which was connected via a channel to a ‘quiet’ pool (Fig. 1). This sound could be either intermittent or continuous, and was broadcasted at different SPLs (one SPL per trial), ranging from a relatively low level (close to ambient sound levels and the hearing threshold) to a relatively high level (Fig. 2).

In Experiment 2, we tested whether search performance of harbour porpoises would be affected by artificial brown noise. The porpoises had to find a thawed dead herring (*Clupea harengus*) hidden inside one of three ‘cages’. Influence of temporal structure and SPL of the sound was investigated by presenting intermittent and continuous sounds at low and high amplitude levels. Furthermore, to investigate whether a change in foraging performance was caused by distraction by the sound, we also presented a ’distraction’

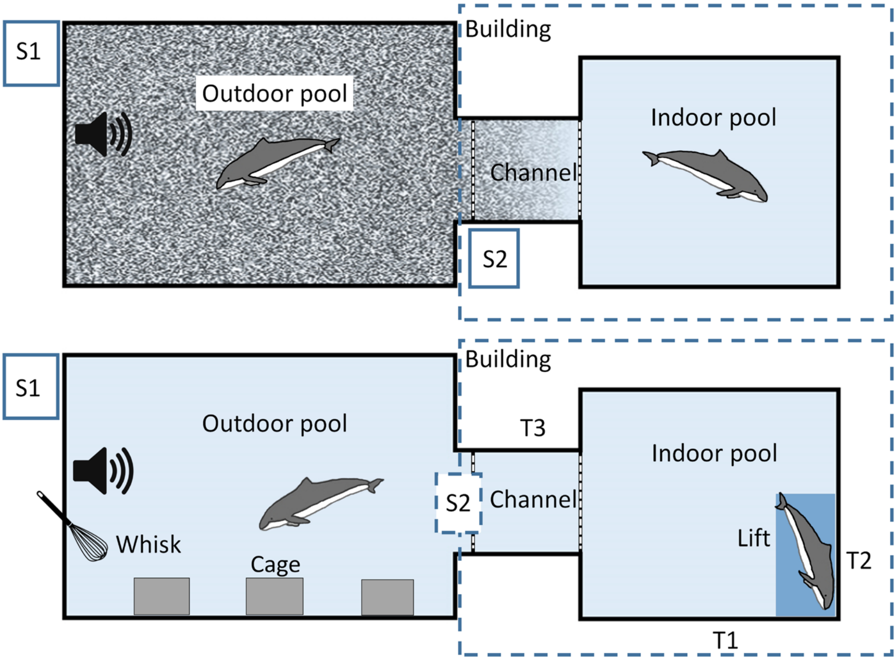

Figure 1: Top-view of the setup for experiment 1 (top) and 2 (bottom). S1 = scientist 1, S2 = scientist 2, T1 = trainer 1, T2 = trainer 2, T3 = trainer 3. Black/white shaded area in top left pool indicates the area with elevated sound levels. Interrupted black and white lines to the left and right of the channel indicate gates that could close off the indoor from the outdoor pool. The interrupted blue line denotes the outer rim of the building in which the right-hand pool was situated. Solid blue lines around S1 and S2 indicate the research cabins in which the scientists were situated (so they were not visible to the animals). Grey rectangles (bottom) denote the fish cages that were used in the food searching task. Note that the whisk seen in the bottom picture was only there for trials in which its effect was tested. In Experiment 2 (bottom), T1 would send one animal to the outdoor pool to search in the food searching task, while T2 lifted the other animal out of the water. T3 controlled the gate to the outdoor pool. Responses were recorded by S2, who was situated on the roof of the indoor pool building, above the channel. Changing of treatments and fish location between trials was done by S1.

stimulus, in the form of a whisk. This object has an unusual echolocation signature, and is known to be an interesting object to the porpoises.

### *Sound stimuli*

Intermittent and continuous sound stimuli were created with artificially generated brown noise using Audacity 2.0.2, and band-passed between 400 and 2000 Hz using Adobe Audition CS6. These frequencies well reflect the dominant frequencies of sounds created by shipping and pile driving and are audible to porpoises, but are at the low end of a harbour porpoise's hearing range, where their hearing is relatively insensitive. It is far below the frequencies at which echolocation clicks are produced (Fig. 2 & 3). Hence, these stimuli allow testing of whether sound, non-overlapping with harbour porpoise echolocation or prey-sounds, can impede foraging performance by inducing avoidance or distraction.

The intermittent stimulus (a repetition of 50 ms pulses every 2 s with a short fade in and

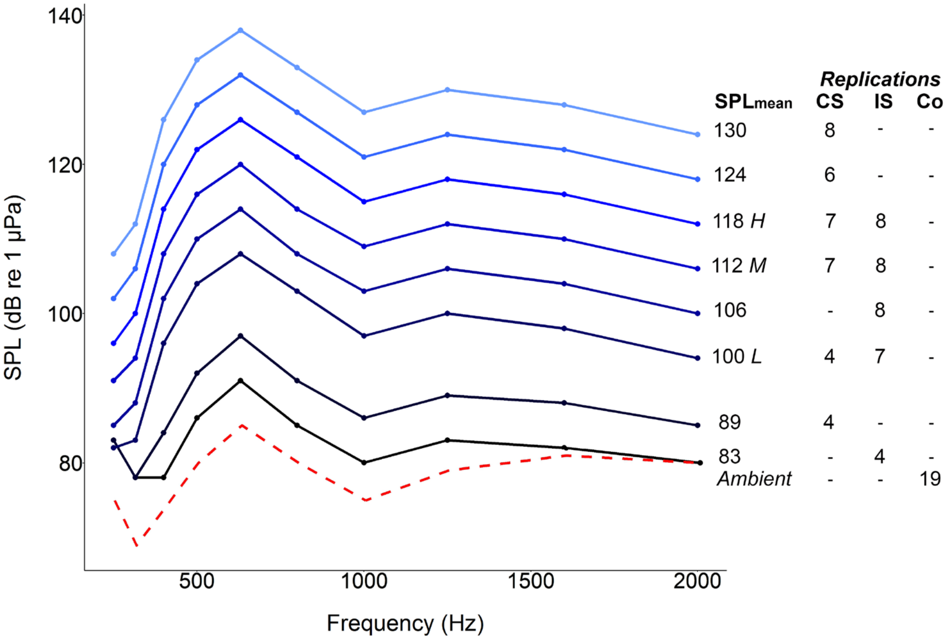

Figure 2: SPLs as measured in the outdoor pool for the played-back sound (depth: 1 m, distance from the transducer: 2 m). Sound bandwidth was 400-2000 Hz. Black to blue solid lines indicate increasing gain levels of the played sound. The normal ambient sound level in the pool is indicated by the red dotted line. SPLs were similar for both intermittent and continuous sound. Also indicated are the average SPL per stimulus, as well as replications for continuous (CS), intermittent (IS) and control (Co) sound types for experiment 1. Three SPLs were shared between sound types, indicated in the figure by "L" (low), "M" (middle) and "H" (high). For Experiment 2, mean SPLs of 100 and 118 dB re 1 µPa were used for 'low SPL' and 'high SPL' treatments, respectively.

fade out to remove onset and offset clicks) was created by adding silent intervals of 150 ms to the continuous stimulus, after which peak-to-peak SPL between the two stimuli was compared and stimuli were equalized until a similar SPL was obtained. The sound files were played from a laptop using LabVIEW Run-Time Engine 2010. The laptop was connected to an underwater transducer (Lubell LL1424HP) via a sound card (National Instruments-USB 6259), a ground loop isolator, a buffer(custom-built), a variable passive low-pass filter (custom-built), a power amplifier (East and West Inc.-LS5002), and an isolation transformer (Lubell AC1424HP). The filters functioned to suppress any electrical noise. The transducer was lowered into the water at least an hour before experiments started, to let the animals get used to its presence. To test for any effects of the experimental set-up not caused by the broadcasted signals, a control playback was conducted using a sound file that contained no sound (silent control).

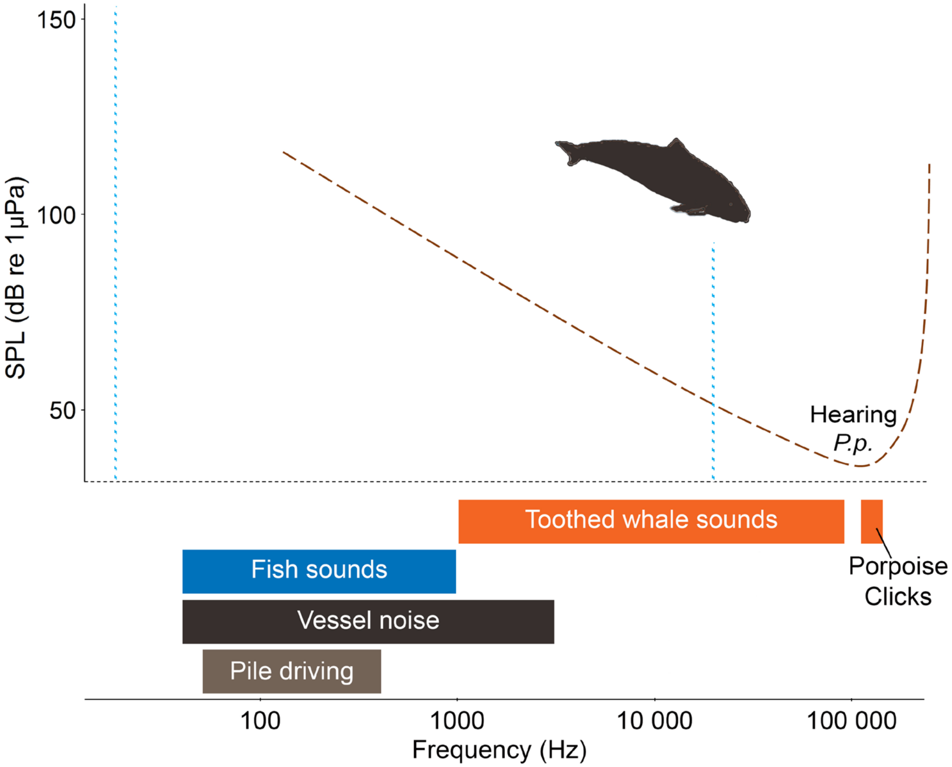

Figure 3: Schematic representation of general frequency bandwidths of pile driving sound (brown-grey; Göttsche et al., 2015; Schecklman et al., 2015), ship sound (black; McKenna et al., 2012; Simard et al., 2016), sounds produced by fishes (blue; Hahn and Thomas, 2008; Kasumyan, 2008; Langård et al., 2008), sounds produced by dolphins (orange) and harbour porpoise echolocation clicks (orange bar to the right of dolphin sounds; Kastelein et al., 2013; Villadsgaard et al., 2007). On top in a brown dashed line is the hearing sensitivity of harbour porpoises (Kastelein, Schop, et al. 2015). As a reference, general human hearing range is shown as blue dotted lines. Note that ship and pile driving sounds, as well as fish sounds, do not overlap with harbour porpoise clicks, but are within their audible range.

### *Experiment 1: Spatial avoidance test*

#### *Experimental procedure*

A trial consisted of 15 minutes of exposure to either intermittent sound, continuous sound, or a silent control. Before exposure, care was taken to remove any additional sound sources, as well as restricting access for everyone other than two researchers. During experiments, the two researchers remained in the playback and recording cabins so they were not visible to the porpoises. The animals were free to move between both pools during the entire exposure period (Fig. 1). Their behaviour was recorded using a camera (Go Pro 3) for the outdoor pool and one for the indoor pool (Conrad 750940). Another camera (Conrad 750940) was used for the outdoor pool as a backup. Two to eight experiments were carried out per day, depending on weather conditions (no trials were conducted during rain or wind >5 Beaufort; under those conditions the test stimuli could have been masked). Intervals between trials were at least 30 minutes, to eliminate possible carry-over effects. Stimuli were presented in a pseudorandom setup, with a minimum of one silent control per day.

#### *Detailed stimuli description*

Intermittent and continuous stimuli started with a 1-min ramp-up (gradual amplitude increase) to ensure that the sound would not cause a startle response, since that was not the purpose of the experiment. The ramp-up was part of the 15-min exposure period. To assess the effect of SPL, stimuli were played at different SPLs in pseudorandom order, ranging from on average 83 dB to 130 dB re 1 µPa with 6-17 dB steps between SPLs (Fig. 2). SPL output levels and ambient SPL were measured on a single day in between the experiments. During the pilot study, both animals seemed to react more strongly to intermittent than to continuous sound. Therefore, the maximum SPL of intermittent sound was lower (118 dB) than for continuous sound (130 dB). Three SPLs were shared between the sound types: 100 dB ("low"), 112 dB ("middle") and 118 dB re 1 µPa ("high").

#### *Behavioural measurements*

For each individual, we recorded the time spent in either pool, the number of transitions between pools, the number of surfacing events and the average speed in the outdoor pool from the video recordings. The video recordings were analysed blind to the exposure condition. In three trials, no Go Pro recordings were available, so the recordings from the backup camera were used. Average speed was calculated by measuring the distance travelled per individual for the second and the last minute of the exposure period (the first minute contained the ramp-up) using tracking software (Kinovea 0.8.15), and dividing by time. To record distance from the video images, in each frame, animals were identified and tracked via the zinc ointment mark placed on their back specifically for tracking purposes (at 15 frames per second). This ensured consistent placement of the position-tracker on the body. The locations per frame (provided by the programme in pixel coordinates) were corrected for fish-eye and perspective distortion and rescaled to location in the pool (x and y cm from two fixed pool edges). Visual examination of the data showed no clear differences in swimming speed between the start and the end of the exposure period, so speed (m/s) was calculated by averaging distance travelled over time for the two 1-min periods, resulting in one average swimming speed per trial. Swimming speed was only calculated for control and trials with SPLs that were shared between sound types. Due to a relatively low quality of the Conrad video recordings, only recordings of the Go Pro camera could be used for measuring speed.

In total, 124 experiments were conducted. Some experiments had to be terminated before the full duration of 15 min. Those experiments were excluded from the analysis. For all response variables except one (number of transitions), exclusion of the shorter experiments did not affect the results. Eventually, at least 4 replicates per treatment were used in the analysis (Fig. 2).

### *Experiment 2: Foraging performance test (food searching task)*

#### *Food searching task training*

The porpoises were trained on a search task for a dead thawed herring ("the fish"), otherwise used as food, inside a cage, made of PVC tubes and a surrounding black net of fine mesh (5 mm mesh size) attached to the inner side of the tube construction. The net only partially obstructed vision, but prevented direct access to the fish. The tubes were perforated, filling them with water for acoustic transparency. The animals were trained separately, by keeping one individual busy while training the other. First, a single cage was used to train the animals to station in front of it to indicate their choice. When successful, the training was expanded to discrimination between two cages, one of which would now be empty. After a sufficiently high success rate (90%), the training was expanded to three cages. Then, the animals were trained to start in the indoor pool and search for the fish in one of the three cages in the outdoor pool (Fig. 1). After each successful attempt, the animal would hear a whistle and would receive a piece of fish as a reward. After an unsuccessful attempt, one of the trainers would knock on the wall of the pool to call the animal back to the trainer (no fish would be given). Animals were trained separately, but on the same day. Total training duration for both animals was 2.5 months.

#### *Experimental procedure*

Every session consisted of 7 trials, in which all stimuli (intermittent low SPL, intermittent high SPL, continuous low SPL, continuous high SPL, silent control, whisk) were presented once in a balanced order. The first trial of the session was always a test trial, to investigate if the animals' motivation was sufficient to continue. To reduce the risk of an animal losing motivation during the experiment, intervals between trials within a session were kept as short as possible. Fish location followed a balanced design, so that every stimulus-cage combination was replicated 4 times per individual. The fish was hung from a wooden plank on top of the cage by a transparent wire to keep its position stable, thus making the detection probability the same per trial. When placing the fish in a cage before each trial, we put a plank on all three cages to minimize any acoustic cues that might arise from putting the plank with the fish on just a single cage.

A trial started with (the tested) animal 1 inside the pool and (the non-tested) animal 2 temporarily lifted out of the water (the animal was trained for this behaviour). This was done to avoid animal 2 being exposed to sound that might leak into the indoor pool. Trainer 1 then signalled animal 1 to 'search'. When animal 1 approached the gate, this was opened, and closed again when animal 1 was through. Animal 1 was then allowed to investigate the three cages and indicate its choice (Fig 1). At the end of the trial, animal 1 was allowed back inside, sound exposure stopped and animal 2 was lowered back into the water. When both animals were inside, the fish was moved to another cage, or lifted from the water and returned to the same cage. This process was repeated until animal 1 had performed 7 trials in succession. Then the animals were switched, so animal 2 would take part in the trials (with a new trial order), while animal 1 was lifted out of the water.

A trial could terminate in four ways. First, animal 1 could choose the correct cage ("Correct"). Researcher 2 would then blow a whistle, and animal 1 would go inside to receive a reward. Second, animal 1 could choose the wrong cage ("False"). Researcher 2 would signal the mistake to trainer 1, who would knock on the pool edge, signalling the mistake to animal 1 and calling it inside. Third, animal 1 could fail to choose a cage within the set time limit of 2 minutes ("Time"). The procedure would then be similar to that of scenario 2. Fourth, animal 1 could go inside without choosing a cage ("No choice"). In this scenario, animal 1 would swim toward the gate, which was then opened, and could go inside. For all types of incorrect response, animal 1 was given a smaller reward than after a correct response (as opposed to the training phase, where no reward was given for an incorrect response). Animal 2, which was being lifted out of the water, would receive a reward after every lift. In some cases, animal 2 would refuse to swim onto the lift, in which case it was asked to keep its head out of the water (such as by touching the trainer's hand).

### *Detailed stimuli description*

Based on the results of Experiment 1, two SPLs were selected from the SPLs used in Experiment 1 and used for the intermittent and continuous sounds of Experiment 2: a relatively low (100 dB) and relatively high average SPL (118 dB) were selected to create 'low' and 'high' sound levels of both sound types (Fig. 2). The whisk was presented with the speaker turned off, hanging roughly 1 m below the water surface on a pole that was a short distance from the edge of the pool (Fig. 1).

### *Behavioural measurements*

All trials were recorded using the outdoor pool video camera (Go Pro), which we scored blind to the exposure condition. For every trial, the response was recorded (correct, false, time or no choice). Search time was measured from the point the animal entered the outdoor pool (body marking was at the edge of the channel opening) to the moment it started to swim back to the gate (measured as the point the animal turned away from the cages and towards the gate, and then swam to the gate).

In total, the animals participated in 33 sessions (male 17 sessions, female 16 sessions) that consisted of multiple trials, so that one session contained all six stimuli. Sessions that did not contain a silent control, because they had to be terminated prematurely, were removed from further analysis. This left a total replication per stimulus of 13 to 14 times for the male and 12 to 13 times for the female. For the male, one session had to be terminated prematurely, but after the silent control was presented and was therefore included in the analysis, leading to one extra replication for the control, intermittent low SPL and the whisk (so 14 replications). For the female, intermittent low SPL and intermittent high SPL were presented twice during one session, thus were replicated 13 instead of 12 times.

### *Statistical analysis*

For Experiment 1, we investigated effects of sound on harbour porpoise spatial behaviour using generalized linear mixed models (GLMM; Bolker et al., 2009). Two models were created, one with time spent inside as the response variable, the other with the total number of transitions between pools as the response variable. In both models, the fixed effects were: SPL, sound type (intermittent, continuous, or control), individual, and the interaction between sound type and individual. The random effect was date. Only SPLs that were present in both intermittent and continuous sound conditions were included in the dataset (100, 112 and 118 dB re 1 μPa), to make a balanced comparison between sound types. To investigate effects of sound on activity, the same fixed and random effects were used in a GLMM with either total number of surfacing events or swimming speed as response variable. A post-hoc Tukey test was performed to compare sound types within an individual. For all response variables, we used the full dataset including all SPLs except ambient to create a separate set of models to test the effect of SPL within the intermittent and continuous sound types (so one model for each sound type). It was not possible to include ambient SPL and investigate the interaction between sound type and SPL at the same time, because the control was, naturally, always presented at the same SPL and would cause singularity in the model and cause a fail. Fixed effects for these models were SPL, individual, as well as interactions between SPL and individual. The random effect was date. Additionally, we created acoustic dose-response curves of both intermittent and continuous sound using the full dataset. Response (yes/no) was defined as spending more time in the quiet pool than the median time spent in the quiet pool during the control. Significance of the dose-response was tested with a generalized linear model (GLM) with binomial distribution. Since the response was compared to the median of all controls, we did not use a mixed model, to avoid grouping of data in the model.

The effect of sound on search accuracy in Experiment 2 was tested using a 2-sample proportion test without continuity correction for percentages of correct responses that seemed to deviate from the control. To test the effect of sound on search time, we performed a GLMM with search time as response variable, and stimulus (intermittent sound, continuous sound, silent control, and whisk), individual, order of the individuals in the experiment and cage containing the fish as fixed factors, including interactions between stimulus and individual, and cage and individual. We included the cage containing the fish to account for variation in search time due to the specific location of each cage. Session number was included as a random effect to correct for nesting of trials within a session. Session correlated strongly with date, so date was not included as a random factor. Like Experiment 1, effect of SPL on the response variable (search time) was tested in a separate GLMM that included as fixed effects stimulus (excluding the whisk), SPL, individual, cage containing the fish, order of the individuals in the experiment, and interactions between SPL and individual, and cage and individual, with session number as random effect.

We performed GLMM analyses using lmer from the package *lme4* and glmmPQL from *MASS* (Venables and Ripley 2002). The GLM was performed using glm from the package *stats* (R Core Team, 2015). The proportion test was performed using prop.test from stats (R Core Team 2015). For swimming speed, a Gaussian distributed model was used. For search time in Experiment 2, a Gamma distribution was used. For the other response variables, we used a quasipoisson distribution to account for the over-dispersion in our data. Due to the use of penalized quasilikelihood by glmmPQL, corrected Akaike Information Criterion (AICc) or similar scores could not be computed, so optimal models were selected using backwards stepwise model selection: starting out with the full model , then stepwise dropping of the most insignificant variable (highest p-value), until all variables left in the model were significant (p-value<0.05; final models: Table 1; Bolker et al., 2009). For swimming speed, model selection was done using dredging (an analysis tool that circumvents the effect of the order of variable removal by testing all possible options; Barton, 2016) and the optimal model was selected using Bayesian Information Criterion (BIC) scores. This score is a more conservative score than AICc and gave higher support for the optimal model than AICc. The results of dredging using BIC concurred with backward selection using F tests with the Kenward-Roger approximation, which we used to calculate p-values (KRmodcomp from package *pbkrtest*; Halekoh and Højsgaard, 2014). Next, we tested validity of the model by plotting the residuals on a 95% confidence interval scale of the expected residuals. All statistics were performed in RStudio (R Core Team, 2015).

## Results

### *Experiment 1: Spatial avoidance test*

#### *Time in quiet pool*

Both animals spent significantly more time in the quiet pool during exposure to both intermittent and continuous sound, than during the control condition (GLMM; both

Table 1: Overview of explanatory variables that were kept in the final versions of each model. Models could either test the effect of sound type (Sound type) or SPL within sound type (SPLintermittent and SPLcontinuous). In the latter case, stimuli that did not include any played sound (silent control and whisk) were excluded from the analysis. Date and session number were random effects used for either Experiment 1 (Date) or Experiment 2 (Session number). All models of Experiment 1 followed a quasipoisson distribution, except for the model on swimming speed, which followed a Gaussian distribution. The models of Experiment 2 followed a Gamma distribution.

| **Response variable** | | **Explanatory variables** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Fixed effects** | | | | | | | **Random effects** | |
| | *effect of* | **Sound type** | **Individual** | **Shared SPLs** | **SPL** | **Cage** | **Treat:Ind** | **SPL:Ind** | **Date** | **Session no.** |
| **Time inside** | *Sound type* | | x | x | | | | | x | |
| | $SPL_{intermittent}$ | | x | | x | | | | x | |
| | $SPL_{continuous}$ | | x | | | | | | x | |
| **Transition** | *Sound type* | x | x | | | | | | x | |
| | $SPL_{intermittent}$ | | x | | x | | | x | x | |
| | $SPL_{continuous}$ | | x | | | | | | x | |
| **Surfacing events** | *Sound type* | x | | | | | | | x | |
| | $SPL_{intermittent}$ | | | | | | | | x | |
| | $SPL_{continuous}$ | | | | x | | | | x | |
| **Swimming speed** | *Sound type* | | x | | | | | | x | |
| **Search time** | *Sound type* | x | x | | | | | | | x |
| | *SPL* | | x | | | | | | | x |

p<0.01; Supplementary Table S1). SPL influenced the time spent inside, with individuals spending more time in the quiet pool with higher SPLs in the noisy pool (Fig. 4). When comparing intermittent to continuous sound for all SPL levels, both individuals spent more time in the quiet pool when exposed to higher SPLs of intermittent sound, but there was no relation between time inside and SPL for continuous sound (GLMM; intermittent: SPL = $p<0.01$; continuous: SPL = p = 0.37). This was also apparent in the dose-response curves: when exposed to intermittent sound, there was a significant effect of SPL for both animals, while for continuous sound no significant dose-response curve could be plotted (GLM intermittent: SPL $p<0.005$; GLM continuous: SPL p=0.77; Fig. 5). 50% response level differed between individuals: for the male, 50% response for intermittent sound was at 93 dB re 1 μPa, while for the female it was at 102 dB re 1 μPa.

### *Pool transitions*

Both animals moved more often between both pools during exposure to continuous sound than during the control (GLMM; continuous: $p<0.05$; Fig. 6; Supplementary Table S2). This was not the case for intermittent sound. SPL did not have an impact

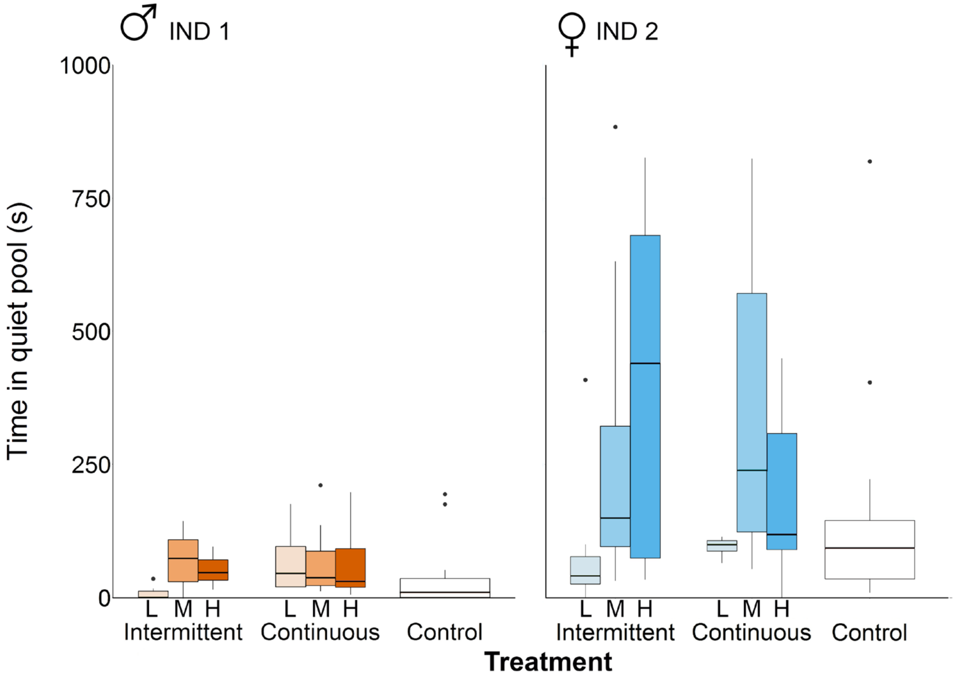

Figure 4: Porpoises spatially avoided noisy conditions. Two captive porpoises – a male (left) and a female (right) – spent time in a quiet pool when exposed to intermittent and continuous sound at three SPLs: 100 (low; L), 112 (middle; M) and 118 dB re 1 μPa (high; H); and ambient sound (Control; 79 dB re 1 μPa). Darker colours indicate a higher SPL (shades of orange for individual 1; shades of blue for individual 2). Boxplots denote 25 to 75 percentiles, whiskers show the 95% Confidence Interval (CI), the black horizontal bar represents the median and dots represent the outliers (data points outside 95% CI). SPL had a significant positive effect on time spent in the quiet pool for both individuals.

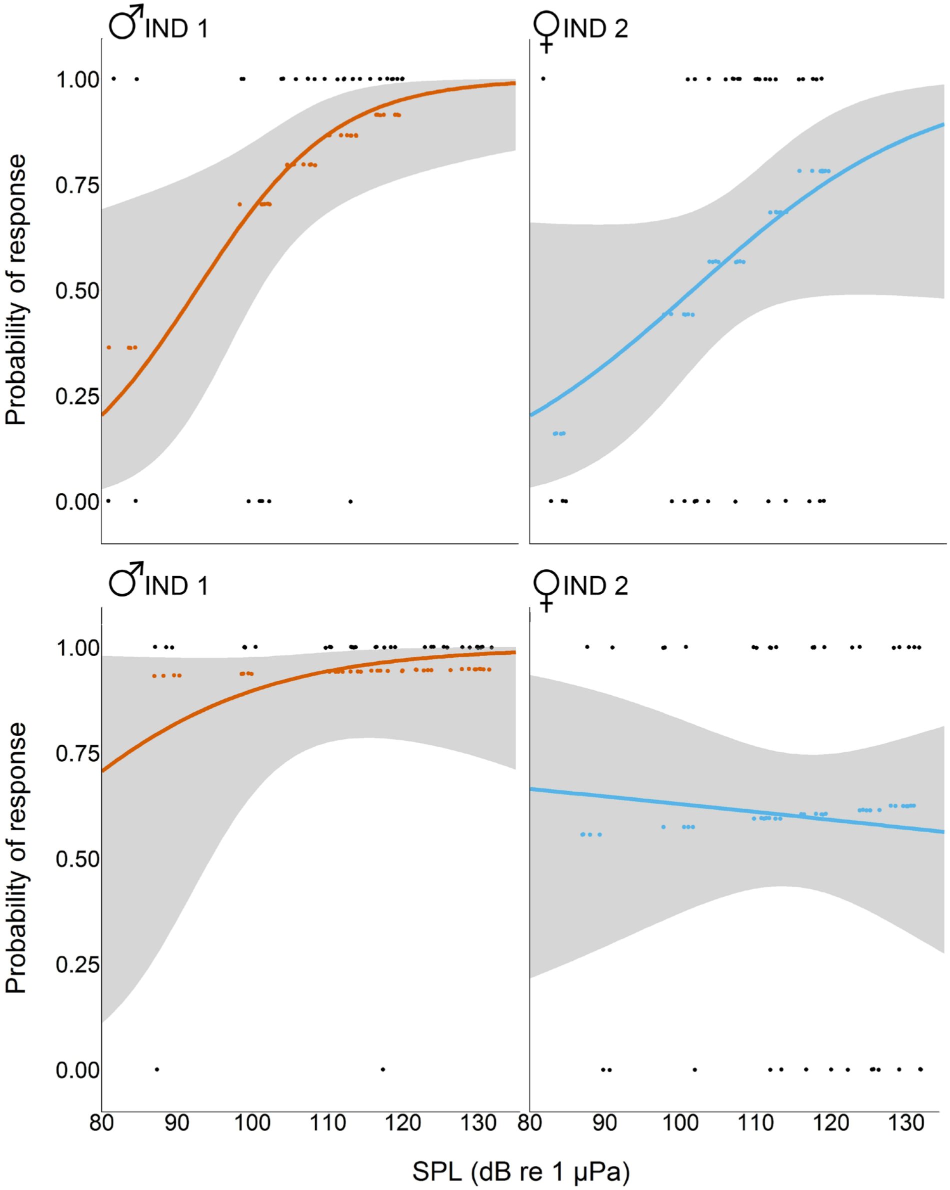

Figure 5: Acoustic dose-spatial response in two captive harbour porpoises, a male (orange, left) and a female (blue, right), to playback of intermittent (top) and continuous (bottom) brown noise of increasing SPL, measured as probability of response (more time in the quiet pool than median of control) during a 15-min exposure period. Dose-response curves are modelled using a generalized linear model with binomial distribution. Output of the model is presented both as optimum curves with 95% confidence intervals (CIs) and as concrete predictions after one run of the model in coloured dots. Actual data are represented with black dots. Note that dots are jittered around their actual value to avoid overlapping in the figure. Further note that for the bottom figures, optimum curves could be drawn in any direction within the 95% CI, indicating that there is no significant effect of SPL in these figures.

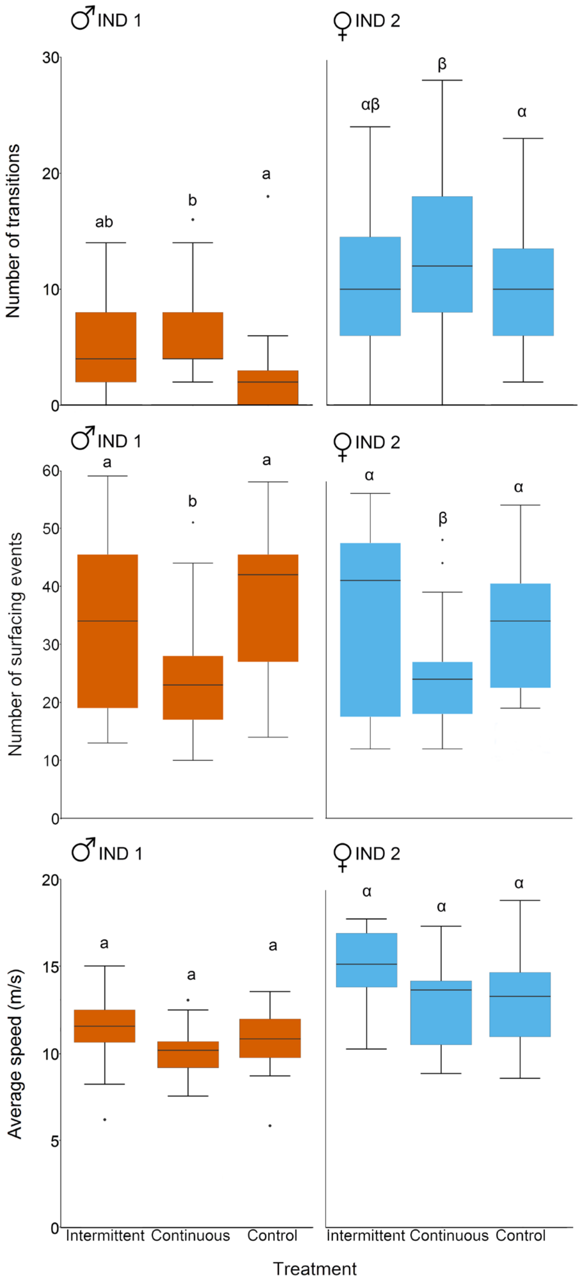

Figure 6: Exploration of behaviour under noisy conditions. Responses of two captive porpoises, a male (orange/dark grey, left) and a female (blue/light grey, right), to three exposure conditions (intermittent, continuous and control) measured as number of transitions between pools during a 15 min-exposure period (top), as number of surfacing events during a 15-min exposure period (middle) and as average swimming speed in the exposure pool over the second and last minute of the 15-min exposure period (bottom). Exposure conditions that differ significantly from each other within an individual are labelled with different letters: a and b for the male and α and β for the female.

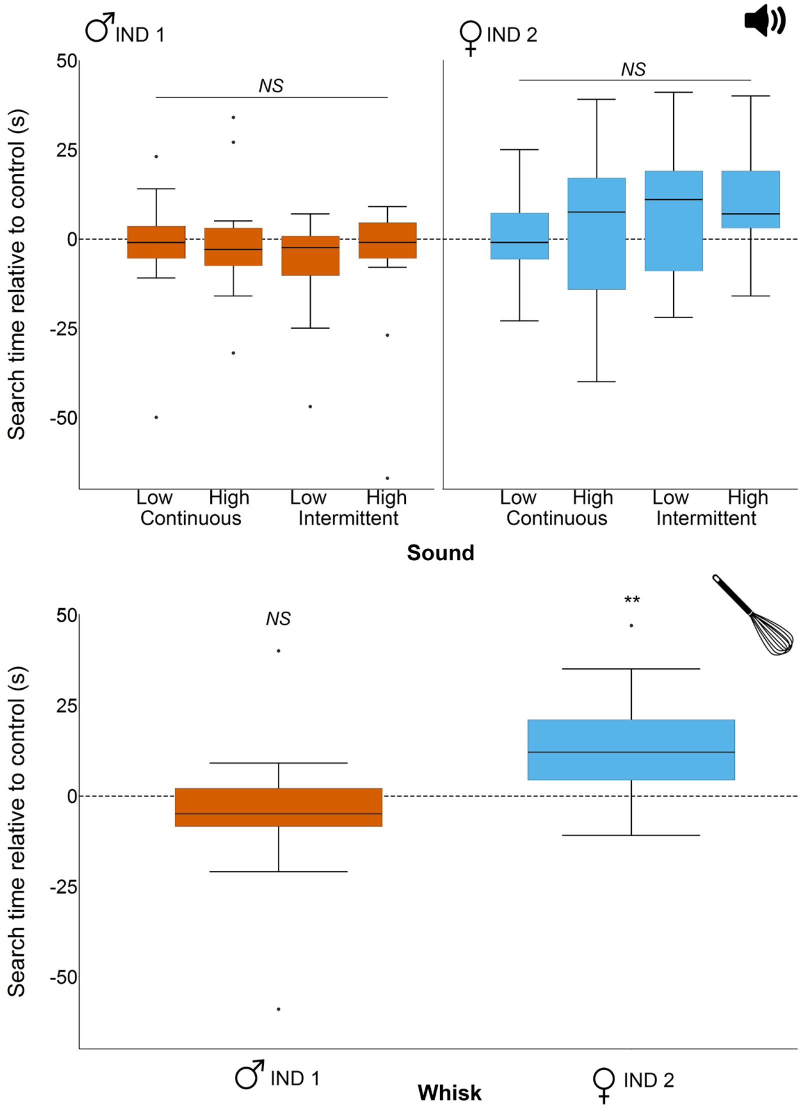

Figure 7: There was no effect of sound or whisk on search time, except for the female to the whisk. Relative search time (s) of two captive porpoises, a male (orange) and a female (blue), for a cage containing a fish in a food search task comprising three cages. Relative search time was calculated by subtracting search time for the control stimulus from search time of the exposure stimulus within the same session of trials. The search could take place under different exposure conditions: control (silence), several sound exposures (top) – continuous sound with high SPL (continuous high), continuous sound with low SPL (continuous low) intermittent sound with high SPL (intermittent high), intermittent sound with low SPL (intermittent low) – or a whisk (bottom). The dotted line indicates where search time does not differ from search time during a silent control. Boxes that are above the dotted line indicate a longer search time, boxes below indicate a shorter search time.

when only the three shared SPLs for both sound types were compared. When all SPLs were included, however, the male moved more often between pools with increasing SPL when the sound was intermittent but not when it was continuous (GLMM intermittent; SPL:individual: $p<0.001$; GLMM continuous; SPL:individual: $p = 0.55$; Supplementary Table S2). This was not the case for the female.

### Surfacing events

Both animals surfaced less during the continuous sound exposure than during both other sound types (GLMM; $p<0.001$; Fig. 6; Supplementary Table S3). Intermittent sound did not influence the surfacing behaviour. Neither did SPL in this model. When comparing all SPLs within sound types, increasing SPL was related to increased surfacing events for continuous sound only in both animals (GLMM continuous; SPL: $p<0.01$, Supplementary Table S3).

### Swimming speed

Both animals showed a non-significant trend – p-value between 0.05 and 0.1 – towards faster swimming when exposed to intermittent sound than during the control (GLMM; intermittent: $p=0.09$; Fig. 6). However, continuous sound did not affect swimming speed (GLMM; continuous: $p = 0.37$, Supplementary Table S4). On average, the female swam faster than the male (27.6 m/s vs. 22.2 m/s, respectively).

## Experiment 2: Foraging performance test (food searching task)

### Correct choices

The male had a high percentage of successful search performances when given the task to find the cage with the fish (all treatments >78%). The female performed correctly 58% of the time when exposed to the whisk, and >74% correctly when exposed to the other treatments. This difference was a non-significant trend (2-sample proportion test: $\chi 2 = 3.56$, df = 1, $p=0.059$).

### Treatment effects on search time

The female, but not the male, was slower in finding the fish when exposed to the whisk (Fig. 7; GLMM; interaction whisk:female $p<0.05$; Supplementary Table S5). Initially, the results showed that the male searched significantly faster under intermittent sound and when exposed to the whisk. This result turned out to be driven by two outliers, both of control trials, in which the male was roughly three times slower in finding the fish than on average. These trials took place during two days in which the male was unusually difficult to motivate for the search task. Therefore, we decided to leave these sessions out of the dataset. After removing outliers, intermittent sound showed a non-significant trend toward shorter search time that was gone in the post-hoc test (GLMM; $p = 0.065$; Tukey pmale = 0.22), although the response of the male was still significantly different from that of the female (GLMM; interaction intermittent:female $p<0.05$). The other stimuli did not have a significant effect. Both ‘Low’ and ‘High’ SPLs did not increase the search time when compared to the control (GLMM; SPL-Low p = 0.47, SPL-High p=0.75, Supplementary Table S5). The cage in which the fish was placed was not retained within the final model, so the location of the fish did not influence the results (GLMM; Supplementary Table S5). The order of the animals – either starting with the search tasking or starting on the lift – was not retained within the final model either (GLMM; Supplementary Table S5).

## Discussion

Harbour porpoises spatially avoided a noisy pool when exposed to intermittent or continuous sound. At the same time, sound did not affect the porpoises’ ability to find a prey in a prey search task. In Experiment 1, both harbour porpoises spent significantly more time in the quiet indoor pool when sound levels were elevated in the outdoor pool. The time the porpoises spent in the quiet pool was also influenced by sound pressure level (SPL): sounds of 100 dB re 1 μPa did not cause a significant increase in avoidance, but SPLs above that did. Interestingly, SPL caused an increase in avoidance for intermittent sound, while avoidance was equal for SPLs above 100 dB of continuous sound. In Experiment 2, we found foraging performance to be affected by a whisk, but unaffected by experimentally elevated sound levels of intermittent or continuous sound at relatively low and high SPLs. In both experiments, the female generally showed a stronger and more variable response than the male.

### *Spatial avoidance of elevated sound levels*

Our experimental study on two captive animals provides evidence that elevated sound levels have the potential to cause area avoidance in harbour porpoises. Motivation to avoid the sound source was supported by the fact that while time inside increased, this was not matched with a consistent increase in swimming speed or movement between the pools, showing that increased time inside could not be explained by increased movement in general (Chapter 2; Kok et al., 2016). Hence, animals were more motivated to spend time in the quiet pool. This conclusion is corroborated by earlier observational studies around wind farm construction sites that indicated a decrease in harbour porpoise presence during construction (e.g. Carstensen et al., 2006).

The concordant patterns with respect to spatial avoidance of anthropogenic sound in captive and free-ranging harbour porpoises are in line with expectations and reports in other taxa. Fishes and other cetaceans avoid areas with high ambient sound levels (Gomez et al. 2016; Neo et al. 2016). In a terrestrial setting, breeding birds have been found to exhibit noise-dependent distribution patterns in density and diversity, while migratory birds have been shown to avoid a phantom road of played back traffic sound (McClure et al. 2013). Also, large mammals, such as grizzly bears (*Ursus arctos*) and elephants (*Loxodonta cyclotis*) have been shown to avoid noisy roads completely or just during day-time (McLellan and Shackleton 1988; Wrege et al. 2010).

The consequences of spatial avoidance due to the presence of an anthropogenic sound will vary with context. Avoidance of an area could reduce foraging opportunities, as harbour porpoises tend to generally stay in areas with medium to high food abundance (Lawrence et al. 2016). The importance of the sound polluted site will determine in part the severity of the effect. For example, in migrating birds, experimentally elevating sound levels near a stop-over area, an important location for the birds, led to avoidance and negatively affected body condition (McClure et al. 2013; McClure et al. 2017). In contrast, harbour porpoises that were exposed to pile driving sound stayed away for 7.5 hours in one area (Tougaard et al. 2009), while leaving another sound polluted area for 3 days (Carstensen et al. 2006). This was possibly related to the importance of the sound polluted area in terms of foraging opportunities (Tougaard et al. 2009). Thus, the importance of the sound polluted area can determine whether avoidance will have small or large consequences for an animal and how long avoidance will last.

Avoidance behaviour in marine mammals has been extensively studied in the context of acoustic deterrent devices (ADDs), or pingers, used in the fishing industry (Teilmann et al. 2006; Kastelein et al. 2008b; Dawson et al. 2013). Harbour porpoises nearly always avoided pingers, both in experimental field studies and in captive studies. However, it is possible that deterrent responses to the same sound stimulus decline over time with repeated exposure (Teilmann et al. 2006). Fading phonophobic response patterns may even reverse when ADDs are attached to nets and potentially become a sort of 'dinner bells' (Carretta and Barlow 2011; Schakner and Blumstein 2013). This suggests that initially aversive, anthropogenic sound may not need to yield permanent avoidance. Accordingly, it has been suggested that also harbour porpoises return relatively soon after an acoustically deterrent event (Scheidat et al. 2011). However, more field studies are needed on swimming patterns for animals that can be identified, for example through individual tagging.

### *Effect of sound conditions on foraging performance*

Although the male did show a non-significant trend toward faster search time for intermittent sound, there was no clear effect of sound on foraging performance in either harbour porpoise. This could be due to the overall variability in search efficiency. Search time for the fish varied from 8 seconds to 93 seconds in trials that led to a correct response in the end. This variability could be related to motivation of the porpoises to participate in the experiment, which was highly variable and subject to weather conditions. It is also likely that the difficulty of finding a fish in our set-up was smaller than finding prey in the wild, which might have reduced the probability of finding a detrimental effect of sound. The whisk, however, did have an effect: it increased search time for the female, although not for the male. This shows that a strong enough disturbance can be a significant distraction in the current set-up, but that the range of sounds included in the experiment were not a strong enough disturbance. This does not exclude the possibility that still higher sound exposure might show a significant effect on foraging performance, but this has to be tested in future studies, taking motivation of the animal, search task difficulty and sample size into account.

### *Effect of temporal structure of sound*

Intermittent sound typically elicited a different response in harbour porpoises than continuous sound. Exploration of movement between the pools, for example, showed that while animals moved more between the noisy and quiet pool when exposed to continuous sound, they did not do so for intermittent sound. So, for continuous sound the animals kept returning to the noisy pool, whereas for intermittent sound animals would stay in the quiet pool and not go back more often than they would during the control. Furthermore, comparison of all SPLs used in Experiment 1 showed that for intermittent sound, an increase in SPL caused an increase in avoidance, while for continuous sound, this relationship was missing. For continuous sound, therefore, SPL functioned as a bimodal indicator: above a certain threshold there was avoidance, below it there was no avoidance.

The differences in response to intermittent and continuous sound could be due to the repeated on- and off-set of intermittent sound, which induces a longer habituation time (Neo et al. 2014) and might increase vigilance more than continuous sound. Most literature to date shows that behavioural and physiological responses to intermittent sound surpasses continuous sound, either in magnitude or duration of the response (Neo et al. 2014; Nichols et al. 2015; Shafiei Sabet et al. 2015; Neo et al. 2016; Radford et al. 2016). In this study, identifying the sound type that elicits the strongest behavioural response is not straightforward. The lack of increased returns to the outdoor pool for intermittent sound suggests possible longer-term avoidance after the sound ceases than would be the case for continuous sound. At the same time, the fact that the porpoises equally avoided a continuous sound regardless of SPL (above a threshold), suggests that porpoises would avoid a larger area around a sound source if it was a continuous sound. Our results comply with a recent meta-analysis of Gomez et al. (2016), who found that in the behavioural effects of sound on cetaceans, context and type of sound were more important than received sound levels (RL) near the animals, even though legislation for mitigation strategies is still based on received level.

Both animals decreased the number of surfacing events during exposure of continuous sound. While this strategy would not result in a reduction of received sound levels (Wensveen et al. 2015), a decrease in surfacing events is a behavioural response observed in potential predator avoidance strategies. Blainville's beaked whales (*Mesoplodon densirostris*), for example, that were exposed to simulated navy sonar and predator vocalisations (killer whales), ascended more slowly from their foraging dives, while also moving horizontally away from the sound source (Tyack et al. 2011). Kastelein et al. (2015b) found opposite results for a captive harbour porpoise exposed to 25 kHz signals that increased respiration rates and jumps, a sign of agitation according to the authors. This indicates that behavioural response may be specific to individuals, context, as well as disturbance type (Visser et al. 2016). Future studies are required to investigate which sound types elicit avoidance, anti-predator responses and agitation in harbour porpoises.

### Individual differences in coping with sound

The two individuals varied in their behaviour and behavioural response to sound. The male generally only showed a small, albeit significant, increase in avoidance and was consistent in its responses. The female, on the other hand, responded highly variably even to the exact same stimulus and showed a much stronger increase in avoidance when exposed to sound above a threshold of 100 dB re 1 μPa. Furthermore, the caretakers reported behavioural differences between the individuals in daily training activities, indicating that the individual differences extend beyond the conditions of this study (L. Helder-Hoek & S. van de Voorde, pers. comm.). These differences could be related to sex, age, experience, personality differences or another feature that has not been measured.

Individual responses to disturbance have been found to vary with factors like coping style, body condition and male-female differences. Zebra fish with pro-active and reactive coping styles showed dissimilarities in cortisol level regulation (a physiological measure of stress level) to repeated netting stress (Tudorache et al. 2013). Eels in poor body condition increased their ventilation rate and decreased their startle response when exposed to a simulated predator under noisy conditions, while eels in good body condition did not (Purser et al. 2016). Moreover, female cichlids (*Neolamprologus pulcher*) were less likely to defend their nest against predators during experimental sound exposure, while males did not change their defensive behaviour (Bruintjes and Radford 2013). Even though these differences in response may impede the extrapolation to large-scale effects, documenting these differences is important, because this knowledge is necessary to predict possible consequences at the population level.

### Conclusion

Two captive harbour porpoises avoided a pool with artificially elevated sound levels. Temporal structure of the sound influenced the effect of SPL: for continuous sound, porpoises equally avoided all SPLs above a threshold, while for intermittent sound there was a relationship between SPL and avoidance.  Even after testing only two individuals, this study gives an indication of possible consequences of anthropogenic sound on foraging behaviour in harbour porpoises and indicates effects of variation in coping style between individuals. Translating the results presented here to a wild population should be done with caution, but it could be that harbour porpoises leave suitable foraging areas due to increased sound levels. Variation between individuals in coping styles when confronted with anthropogenic sound might result in differential selection pressures, potentially changing population structure. In conclusion, we believe our study indicates possible effects of elevated sound levels and we hope will stimulate and guide further investigations of anthropogenic acoustic disturbance.

## Acknowledgments

We are indebted to Manon Horvers, Kysha Kraaijvanger and Rosanna Toma for helping us with the data collection at SEAMARCO, Erwin Jansen of TNO for performing the calibration measurements, Frans Rodenburg, Harald van Mil, Karan Odom and Saskia Klumpers for advice on the statistics and the department of Behavioural Biology at Leiden University for providing feedback on the experimental set-up. Funding was provided by LUF (grand number 2015-12), Gratama Stichting and the Dutch Ministry of Infrastructure and the Environment.

## Appendix

Table S1: Two harbour porpoises avoided a pool with elevated sound levels. Sound levels varied in temporal structure and SPL. In the final models, time spent in the quiet pool was set as response variable, with sound type, shared SPL (SPLs that were used for both intermittent and continuous sound) and individual as fixed effects for the model testing the effect of temporal structure of sound (Sound type), and SPL and individual as fixed effects for the models testing the effect of amplitude within sound type (SPL). Date of the experiment was set as random effect for both models. Significant positive effects are denoted with an upward arrow. The control sound (for model Sound type) and male individual were in the intercept and served as the baseline reference for the other effect levels.

| Model | Effects | Estimate+SE | Df | T | P | Variance+s.d. |
|---|---|---|---|---|---|---|
| Sound type | *Fixed* | | | | | |
| | Intercept | 1.616+0.565 | 96 | | | |
| | SPL | ↑ 0.020+0.005 | 96 | 4.03 | <0.001 | |
| | *Harbour porpoise* | | | | | |
| | Female | ↑ 1.517+0.180 | 96 | 8.403 | <0.0001 | |
| | Random | | | | | |
| | Date | | | | | 0.66+8.56 |
| SPL | | | | | | |
| Intermittent | *Fixed* | | | | | |
| | Intercept | -0.083+1.231 | 51 | | | |
| | SPL | ↑ 0.0328+0.011 | 115 | 2.963 | <0.01 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 1.877+0.209 | 51 | 8.977 | <0.0001 | |
| | *Random* | | | | | |
| | Date | | | | | 0.67+6.97 |
| Continuous | *Fixed* | | | | | |
| | Intercept | 3.345+1.078 | 52 | | | |
| | SPL | 0.008+0.009 | 52 | 0.899 | 0.373 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 0.989+0.198 | 52 | 5.000 | <0.0001 | |
| | *Random* | | | | | |
| | Date | | | | | 0.57+8.89 |

Table S2: Two harbour porpoises moved more between a quiet and noisy pool when exposed to continuous, but not intermittent, sound. Results were obtained using GLMMs with a quasipoisson distribution. In the final models, transitions between the pools was set as response variable, with sound type and individual as fixed effects for the model testing the effect of temporal structure of sound (Sound type), and SPL, individual and the interaction between SPL and individual as fixed effects for the models testing the effect of amplitude within sound type (SPL). Date of the experiment was set as random effect for both models. Significant positive effects are shown with an upward arrow, significant negative effects are shown with a downward arrow. The control sound (for model Sound type) and male individual were in the intercept and served as the baseline reference for the other effect levels.

| **Model** | **Effects** | **Estimate+SE** | **Df** | **T** | **P** | **Variance+s.d.** |
|---|---|---|---|---|---|---|
| Sound type | *Fixed* | | | | | |
| | Intercept | 1.346+0.155 | 95 | | | |
| | Sound type | | | | | |
| | Continuous | ↑ 0.376+0.158 | 95 | 2.381 | <0.05 | |
| | Intermittent | 0.160+0.151 | 95 | 1.060 | 0.292 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 0.911+0.129 | 95 | 7.078 | <0.001 | |
| | *Random* | | | | | |
| | Date | | | | | 0.23+1.76 |
| SPL | *Fixed* | | | | | |
| Intermittent | Intercept | -4.688+1.794 | 50 | | | |
| | SPL | ↑ 0.056+0.016 | 50 | 3.493 | <0.005 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 7.573+1.875 | 50 | 4.040 | <0.005 | |
| | Interactions | | | | | |
| | SPL:Female | ↑ -0.060+0.017 | 50 | -3.520 | <0.001 | |
| | *Random* | | | | | |
| | Date | | | | | 0.30+1.44 |
| Continuous | Intercept | 1.146+0.688 | 52 | | | |
| | SPL | 0.007+0.006 | 52 | 1.148 | 0.256 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 0.745+0.153 | 52 | 4.88 | <0.0001 | |
| | *Random* | | | | | |
| | Date | | | | | 0.10+1.90 |

Table S3: Two harbour porpoises surfaced less when exposed to continuous, but not intermittent, sound. Results were obtained using GLMMs. In the final models, number of surfacing events was set as response variable, with sound type and individual as fixed effects for the model testing the effect of temporal structure of sound (Sound type), and SPL as fixed effect for the models testing the effect of amplitude within sound type (SPL). Date of the experiment was set as random effect for both models. Upward arrows indicate significant positive effects, downward arrows indicate significant negative effects. The control sound (for model Sound type) and male individual were in the intercept and served as the baseline reference for the other effect levels.

| **Model** | **Effects** | **Estimate+SE** | **Df** | **T** | **P** | **Variance+ SD** |
|---|---|---|---|---|---|---|
| Sound type | *Fixed* | | | | | |
| | Intercept | 3.523+0.082 | 96 | | | |
| | Sound type | | | | | |
| | Continuous | ↑ -0.261+0.077 | 96 | -3.382 | <0.005 | |
| | Intermittent | -0.027+0.062 | 96 | -0.432 | 0.67 | |
| | *Random* | | | | | |
| | Date | | | | | 0.31+1.53 |
| SPL | | | | | | |
| Intermittent | *Fixed* | | | | | |
| | Intercept | 3.240+0.390 | 52 | | | |
| | SPL | 0.002+0.004 | 52 | 0.526 | 0.60 | |
| | *Random* | | | | | |
| | Date | | | | | 0.40+1.33 |
| Continuous | *Fixed* | | | | | |
| | Intercept | 2.162+0.440 | 53 | | | |
| | SPL | ↑ 0.010+0.004 | 53 | 2.711 | <0.01 | |
| | *Random* | | | | | |
| | Date | | | | | 0.24+1.60 |

Table S4: Sound exposure did not influence swimming speed of two captive harbour porpoises (Experiment 1). Results were obtained using a LMM. In the final model, swimming speed in the outdoor pool was set as response variable, with individual as fixed effect, and date of the experiment as random effect (BIC = 677.2). Upward arrows indicate significant positive effects. The control sound and male individual were in the intercept and served as the baseline reference for the other effect levels.

| **Model** | **Effects** | **Estimate+SE** | **Df** | **T** | **P** | **Variance+SD** |
|---|---|---|---|---|---|---|
| Sound type | *Fixed* | | | | | |
| | Intercept | 22.666+0.858 | | | | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 5.206+0.712 | 95 | 7.316 | <0.0001 | |
| | *Random* | | | | | |
| | Date | | | | | 9.502+3.083 |

Table S5: Sound exposure did not have an effect on search time in a food searching task for two captive harbour porpoises (Experiment 2). Exposure to a distraction treatment (a whisk) did influence search time for one of the individuals. Results were obtained with a GLMM that followed a Gamma distribution. In the final model, search time in the food searching task was set as response variable, with treatment, individual and the interaction between treatment and individual as fixed effects for the model testing the effect of temporal structure of sound and the whisk (Sound type), and SPL and individual as fixed effects in the model testing the effect of SPL (SPL). For both models, the session within which a trial was conducted was included as a random effect. Upward arrows denote significant positive effects. The control sound and male individual were in the intercept. These served as the baseline reference for the other effect levels.

| **Model** | **Effects** | **Estimate+SE** | **Df** | **T** | **P** | **Variance+s.d.** |
|---|---|---|---|---|---|---|
| Sound type | *Fixed* | | | | | |
| | Intercept | 3.128+0.146 | 113 | | | |
| | Sound type | | | | | |
| | Continuous | -0.013+0.147 | 113 | -0.087 | 0.93 | |
| | Intermittent | -0.270+0.145 | 113 | -1.860 | 0.065 | |
| | Whisk | -0.266+0.166 | 113 | -1.598 | 0.11 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 0.494+0.207 | 22 | 2.390 | <0.05 | |
| | *Interactions* | | | | | |
| | Continuous:Female | ↑ 0.093+0.206 | 113 | 0.450 | 0.65 | |
| | Intermittent:Female | ↑ 0.466+0.204 | 113 | 2.289 | <0.05 | |
| | Whisk:Female | 0.531+0.235 | 113 | 2.254 | <0.05 | |
| | *Random* | | | | | |
| | Session number | | | | | 0.29+0.40 |
| SPL | *Fixed* | | | | | |
| | Intercept | 3.011+0.119 | 93 | | | |
| | SPL | | | | | |
| | Low | -0.075+0.103 | 93 | -0.726 | 0.47 | |
| | High | 0.033+0.103 | 93 | 0.321 | 0.75 | |
| | Harbour porpoise | | | | | |
| | Female | ↑ 0.740+0.132 | 22 | 5.615 | <0.0001 | |
| | *Random* | | | | | |
| | Session number | | | | | 0.26+0.40 |

# Diving apart together:
# Vertical call propagation in long-finned pilot whales

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*Submitted to Journal of Experimental Biology*

## Abstract

Group-living animals must communicate to stay in contact. In long-finned pilot whales, there is a trade-off between the benefits of foraging individually at depth and the formation of tight social groups at the surface. Using theoretical modelling and empirical data of tagged pairs within a group, we examined the potential of pilot whale social calls to reach dispersed group-members during foraging periods. Both theoretical predictions and empirical data of tag pairs showed a potential for communication between diving and non-diving group members over separation distances up to at least 385 m (empirical) and 1800 m (theoretical). These distances are at or exceeding pilot whale dive depths recorded across populations. Call characteristics and environmental characteristics were analysed to investigate determinants of call detectability. Longer calls with a higher sound pressure level (SPL) that were received in a quieter environment were more often detected than their shorter, lower SPL counterparts within a noisier environment. In a noisier environment, calls were louder and had a lower peak frequency, indicating mechanisms for coping with varying conditions. However, the vulnerability of pilot whales to anthropogenic noise is still of concern, as the ability to cope with increasing background noise may be limited. Our study shows that combining propagation modelling and actual tag recordings provides new insights into the communicative potential for social calls in orientation and reunion with group members for deep-diving pilot whales.

## Introduction

Communication among members of social groups is an essential element of their social behaviour, allowing them to share information, find mates, defend territories, recognise offspring, and many other life functions (Kondo and Watanabe 2009; Ladich and Winkler 2017; Snijders and Naguib 2017). Group living can provide safety from predators (Lehtonen and Jaatinen 2016), enhance foraging efficiency (e.g. local enhancement, group-hunting: Thiebault et al. 2014; Herbert-Read et al. 2016) and create opportunities for reproduction (e.g. aggregating and lekking, social breeding: Ryder et al. 2009). However, group-living requires a way of communicating that allows associated animals to maintain or re-establish contact, particularly for wide-ranging animals. A powerful sensory modality to keep track of group members is the hearing of each other's sounds, especially if distances between individuals become greater or visibility is limited (Kondo and Watanabe 2009).

Odontocetes, or toothed whales, are sound-oriented animals that typically live for long periods in the same social groups. Group-stability ranges from short-term, fission-fusion societies in some species to long-term, stable matrilineal groups in others (Whitehead et al. 1991; Connor et al. 1998; Ottensmeyer and Whitehead 2003; Hartman et al. 2008). As lights attenuates rapidly in water, odontocetes predominantly use sound for orientation, foraging, and social communication. Odontocetes use social calls for a wide variety of functions, including recognition and relocation of close associates (Deecke et al. 2010; Cantor et al. 2015). Bottlenose dolphins (*Tursiops truncatus*), for example, use individual-specific vocalisations to identify and relocate specific group members (Janik and Slater 1998).

Interestingly, some deep-diving odontocete species that live in the most stable groups, such as sperm whales (*Physter macrocephalus*) and pilot whales (*Globicephala spp.*) do not seem to coordinate hunting effort but typically catch prey individually at depth (Whitehead et al. 1998; Ottensmeyer and Whitehead 2003; Watwood et al. 2006; Visser et al. 2014; but see Aoki et al. 2013 for an exception). Foraging sperm whales, for example, form rank formations, potentially to avoid targeting the same prey (Whitehead 1989). Foraging at depth can therefore lead to spatial separation between group members, which must be relocated when individuals return to the sea surface. Acoustic relocation may be possible through acoustic tracking of echolocation cues from group members and/or directed acoustic signalling through social calls (Parks et al. 2014). Long-finned pilot whales produce more social calls during foraging bouts, when individuals are dispersed, than during non-foraging periods when individuals are several body lengths apart (Visser et al. 2017). However, it is still unknown if their social calls during foraging have the capacity to effectively transmit information between deep-diving and shallow-diving or surfacing group members, or which call features facilitate communication over larger distances.

Besides distance, call detectability can be influenced by the ambient noise level at the receiver and by call characteristics (Brumm and Slabbekoorn 2005; Wiley 2013). Both natural and anthropogenic sources can increase the ambient noise level, leading to a reduction in the range of call detectability (Janik 2000; David 2006; Jensen et al. 2009). Call characteristics that may enhance detection include long duration, high call amplitude, a signal bandwidth that does not overlap with ambient noise, a caller orientation directed to the receiver and low peak frequency (Brumm and Slabbekoorn 2005). These can be tuned by the producer and thereby adapted to situations with poor conditions for call detection. For example, in response to anthropogenic sound, killer whales produced longer calls than in low ambient noise conditions, potentially facilitating detection (Foote et al. 2004).

Propagation of marine mammal calls has mostly been studied in horizontal contexts, because species living in shallow waters are more easily studied and are likely to be limited by horizontal rather than vertical propagation distance (Janik 2000; David 2006; Miller 2006). However, horizontal and vertical propagation are not easy to compare. Horizontal call propagation can be heavily influenced by the sound speed profile and boundary effects from the water surface and the bottom in shallow waters, which creates multiple acoustic pathways (Marsh and Schulkin 1962). These boundary effects are less complex for vertical propagation, because of the steep angles at which the sound travels through the water column and interacts with the surface boundary. At (near) vertical angles, the sound undergoes little refraction or reflection when it crosses thermoclines, thus keeping only one acoustic pathway (Ainslie 2010).

Call propagation can be determined empirically, by measuring a call at the producer and at the receiver (Piza and Sandoval 2016), or theoretically, by calculating transmission loss of the call (Mercado III and Frazer 1999; Miller 2006; Jensen et al. 2012). Up till now, studies on marine mammal call propagation relied on theoretical modelling, because of the difficulties involved in measuring calls at both the producer and receiver at larger spatial scales on free-ranging whales in the marine environment. Here, we overcome this limitation by applying suction-cup attached sensors, which record sound, movement and depth, on multiple individuals in one group (Pasquaretta et al. 2015; Palmer et al. 2017; Snijders et al. 2017).

We examined relocation of close associates through acoustic communication and reception of social calls in a deep-diving odontocete: the long-finned pilot whale (*Globicephala melas*, Traill 1809). Using a combined theoretical and empirical approach, we tested 1) whether pilot whales have the theoretical potential to communicate when vertically dispersed, 2) whether calls produced by or close to a tagged whale were detectable by humans in recordings of tags attached to a potential receiver and 3) how call detectability was influenced by call characteristics and the acoustic environment at the receiving whale. We hypothesize that pilot whales have evolved social calls with transmission properties adapted for acoustic communication over spatial scales that allow for communication and reunion with dispersed group members during and following foraging dives. Environmental noise could hamper this potentially critical relocation process, but its effect might also be mitigated to some extent by potential signal adjustments of the sound-producing animals.

## Materials and methods

### *Theoretical call propagation and detection*

To explore the theoretical detection range of deep diving pilot whale social calls, we adopted a propagation model in RStudio (version 3.5.2) to simulate vertical propagation in deep waters, based on the assumption of spherical spreading (Urick 1983):

$$RL = SL - 20\log_{10}(R/1m)\ \mathrm{dB} - \alpha R \qquad (1)$$

Here, $RL$ = received level (dB re 1 μPa), $SL$ = source level (dB re 1 μPa·m), $R$ = distance between producer and receiver (m) and $\alpha$ = frequency-specific attenuation (Ainslie and McColm 1998) (dB/m). Boundary effects such as Lloyd's mirror effect were ignored, because we focused on vertical transmission, in which surface reflections will be very limited. Furthermore, reflected waves of different frequencies will have alternating interference patterns, leading to an overall neutral effect on signal strength of a broadband signal (Jensen et al. 2011). Pilot whale social calls are typically composed of frequency modulated tonal calls with harmonics, also termed whistles (Visser et al. 2017). Individuals therefore can potentially perceive calls through multiple frequency channels spread over a wide bandwidth (3-9 kHz as found by Rendell et al. 1999).

Whether an animal detects a call depends on whether the received level is above the hearing threshold, the signal-to-noise ratio in the presence of a masking sound, and the orientation of the animal relative to the masker (Erbe et al. 2016). Perhaps counterintuitively, detection of tonal signals is not yet possible when signal and noise at the same frequency are equal in sound level. Detection of tonal sounds only occurs when there is a slightly higher level of the signal compared to the noise (Moore 2013). This minimal signal-to-noise ratio at which an animal can detect a sound, the critical ratio (CR; Fletcher 1940; Erbe et al. 2016) is frequency dependent. It is defined as the minimal level difference in sound pressure (SPL) of the signal and the level of the power spectral density of the root-mean-square sound pressure (PSD) of the masking noise that is required for hearing. Therefore, the modelled detection per frequency was based on received level, critical ratio and ambient noise level.

As critical ratios for long-finned pilot whales have not been measured, the model was based on average critical ratios from other delphinids, at 18, 23 and 28 dB re 1 Hz for 1, 5 and 20 kHz signals respectively (Erbe et al. 2016). Ambient noise levels were taken from the acoustic recordings of the suction-cup attached archival tags (DTAG-2, SOUNDTAG lab, University of St Andrews, United Kingdom), used on the tagged pilot whales for the empirical data collection. The PSD of ambient noise was measured within one-third octave frequency bands (10-base, also referred to as decidecade). The average PSD was computed by dividing the one-third octave band SPL by the frequency bandwidth in Hz over which they were measured, corrected for flow noise by taking the level of correlated noise as the ambient noise level (von Benda-Beckmann et al. 2016). After comparison of the mean and median of the PSD measurements, we took median and 25 and 75 percentiles to describe the general variation in the noise levels, because the median was least sensitive for outliers in the data. At 20 kHz, ambient noise levels could not be reliably measured due to high levels of system noise. Instead, a typical ambient noise level at 20 kHz for sea state 2 (highest sea state in which data were collected) was used (Wenz 1962).

Calls were considered to be detectable by the pilot whales when the received level (RL) within a frequency band exceeded the level of ambient noise mean spectral density plus the critical ratio. When the masking sound source is at a different direction than the calling animal, spatial masking release can reduce the degree of masking (e.g. Erbe et al. 2016). Since the focus of this study was on relatively low frequency calls with limited masking release (Au and Moore 1984; Brumm and Slabbekoorn 2005), we did not control for masking release, which provided a conservative measure of the detectability of the calls.

Long-finned pilot whales produce social calls with varying peak frequencies (frequency with highest SPL; Taruski 1979; Visser et al. 2017). The main range of peak frequencies was determined from an empirical dataset. The minimum (1 kHz), maximum (20 kHz) and mean peak frequency (5 kHz) were used to model call detectability. The used source levels were based on levels found for short-finned pilot whales, 145-160 dB re 1 μPa·m (Turl and Fish 1976), which are in the range of reported source levels for other odontocetes (Janik 2000; Miller 2006). The source levels were verified by calculating source levels from a subset of calls collected from one of the tag pairs. The 1/3-octave band SPL at the peak frequency was back-propagated, assuming spherical spreading and accounting for frequency-dependent absorption (likely to have a small effect at these distances). Mean (s.d.) source levels found were 146.5 (9.5) dB re 1 $\mu Pa^2m^2$, which fell within the range of earlier reported source levels (Fig. 1).

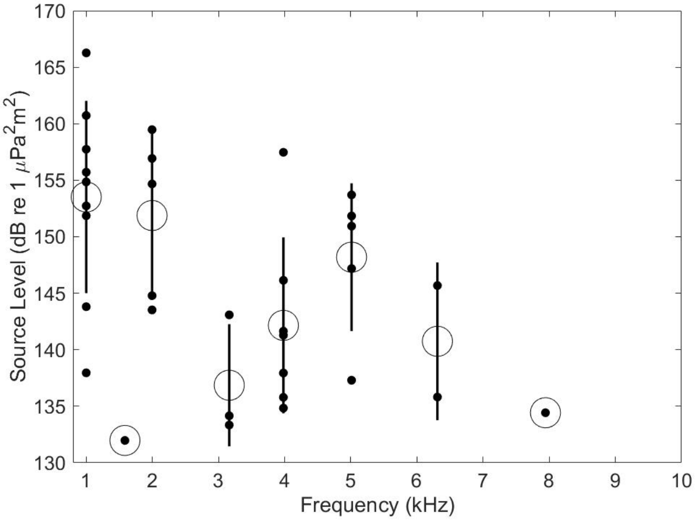

Figure 1: Back-propagated source levels calculated from 1/3 octave peak frequency bands (N = 33). Blue dots represent individual calls, open circles are means per frequency band and vertical lines represent standard deviation.

## *Empirical call propagation and detection*

### *Ethical statement*

All research activities were carried out under permits issued by the Norwegian Animal Research Authority (permit no. S2011/38782), in compliance with ethical and local use of animals in experimentation. The research protocol was approved by the University of St Andrews Animal Welfare and Ethics Committee and Woods Hole Oceanographic Institution's Animal Care and Use Committee.

### *Data collection*

Pilot whale social call and dive data were collected using DTAGs in 2009-2010, off Lofoten, Norway, as part of a larger project to study the effects of naval sonar on cetaceans (3S project) (Miller et al. 2012). All data used in this study were recorded during baseline pre-exposure periods. Tagged whale pairs were always two individuals from the same group (Visser et al. 2014). Dive depth was sampled every second. Acoustic recording sampling frequency was 192 kHz, except for tag 138a (96 kHz), with 16-bit resolution and sensitivity of -189±3 dB (mean ±s.d.) (Johnson and Tyack 2003). The acoustic tags were calibrated in an anechoic tank prior to the experiments (Wensveen 2016).

### *Call selection*

Calls were selected using a step-wise procedure. First, acoustic recordings were analysed by two independent observers who visually marked high amplitude calls from the recordings (amplitude colouration close to the maximum of the fixed colour scale and higher on the scale than most other sounds). All recordings were analysed with identical spectrogram settings: colour scale limits = -90 – 0 dB; FFT block size = 512; Hamming window; overlap = 256; display length = 15 s. High amplitude calls were considered to be calls produced by the tagged whale or an individual in its close vicinity (Alves et al. 2014).

Second, a subset was created with calls with a signal-to-noise ratio (SNR) of ≥10 dB at the producer to minimize measurement errors due to the ambient noise (sensu Jensen et al. 2011). The SNR level was calculated as call-SPL minus total ambient noise-SPL (uncorrected for flow noise, since all noise could influence the acoustic measurements) at the one-third octave band around the peak frequency of the call. To determine the peak frequency of the call, the PSD was measured for one-third octave frequency bands. Peak frequencies were identified in the spectrogram as the frequencies with maximum amplitude in the PSD of the pulse using the entire pulse duration as integration time. The peak third octave was also used to measure the PSD in 200 ms of ambient noise within 4 s before or after a call (the ambient noise section). The period of 200 ms concurs with the typical aural integration time of marine mammals (Kastelein et al. 2010; Erbe et al. 2016).

### *Call detection*

Acoustic recordings of paired tags were time-synchronised, correcting for clock-drift by averaging travel times of clicks produced on tag A and received on tag B, and for near simultaneously produced clicks on tag B and received on tag A (*DTAG* toolbox, Johnson 2005). Clock-drift was then determined as the time-difference from the mean travel time. Clicks produced by the tagged whale were determined from the angle of arrival of the click on the tag, calculated from the difference in time of arrival between the two tag hydrophones. Hence, the precise clock-drift could only be determined for two out of three tag pairs. For the other tag pair, time-synchronisation was less accurate, but still accurate enough (error in order of milliseconds) to link produced and received calls.

Detectability of the selected calls of one tag on the other tag was scored visually per call (Fig. 2). A call was considered detected if the call contour could be reliably identified on the spectrogram of the receiving tag. Harmonics did not have to be visible for reliable identification, but often were. Calls that were produced while the receiver whale was surfacing were excluded, because of the high level of flow noise and surface splashes that typically coincide with the surfacing event. Detectability was scored as 1 (detectable) or 0 (undetectable). As calls were often produced in distinct sequences, the position of the produced call relative to other calls was used to check whether no detectable call was missed in the analysis.

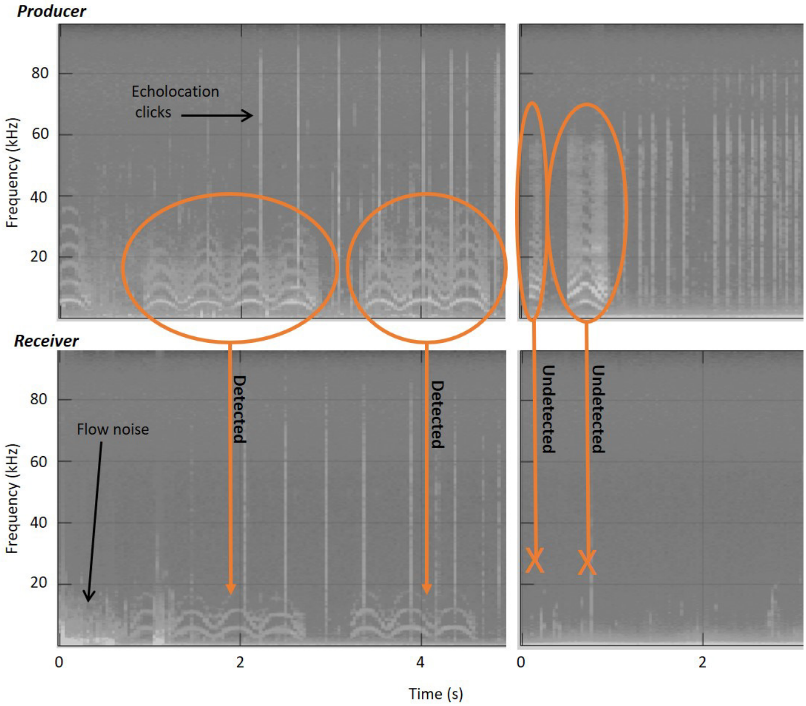

Figure 2. Time-synchronised spectrograms of recordings on producer and receiver tags showing examples of detected (left) and undetected (right) calls. Note that the detected calls are longer than the undetected calls, which makes the noise at the start of the recording less influential for detection.

### Noise filtering

Ambient noise sections and calls were filtered to exclude echolocation clicks using a custom-built script (Supplements; Miller et al. 2012). This procedure was undertaken post analysis of call detection, so that the acoustic background when detecting calls would be similar to the acoustic background experienced by the whales. A click was detected when the rise between two subsequent root-mean-square levels (of a centred moving average with a window length of 2 and 10 ms, respectively) in the >30 kHz frequency range was ≥3 dB. Start and end of a click was found using a third RMS level, with a window length of 6 ms. Each detected click was then replaced with zeroes in the pressure wave form.

We explored the ambient noise in the 0-7 kHz frequency range for every section to investigate whether ambient noise recordings were dominated by flow noise (*sensu* von Benda-Beckmann et al. 2016). Flow noise can be measured by differentiating the noise that is similar between the two hydrophones of the tag (correlated noise) and noise that is not (uncorrelated noise). Only correlated noise is likely to be part of the ambient noise that surrounds the tagged animal, while uncorrelated noise is created by flow noise. Calls at the producer tag for which correlated noise was <6 dB greater than uncorrelated (flow) noise were considered to be influenced too much by flow noise and were removed from the dataset.

### Call characteristics

For each selected call, we recorded production time, peak frequency, PSD at peak frequency, duration (all on producer tag), call arrival time at receiver tag and PSD of total ambient noise at producer and at receiver tag. Call duration was then taken as the 90% energy contour of the call, using a 1-50 kHz band-pass 4-pole Butterworth filter. For detected calls, ambient noise level at the receiver was measured within 4 s surrounding the received calls. For undetected calls, ambient noise level at the receiver was measured at the time the call would have been received. For detected calls, we calculated the signal excess above the ambient noise as:

$$SE = RL - DT \tag{2}$$

With

$$DT = NL - 10\log_{10}(bandwidth)\ \mathrm{dB} + CR \tag{3}$$

Where SE = signal excess (dB), RL = received SPL of the call at peak frequency (dB re 1 μPa), DT = detection threshold (dB re 1 μPa), NL = total ambient noise level at receiver at call peak frequency (dB re 1 μPa), bandwidth = bandwidth of third octave level around peak frequency (Hz) and CR = critical ratio for peak frequency (dB re 1 Hz), taken from the average critical ratio found for odontocetes (Erbe et al. 2016).

### *Vertical distance*

Vertical distance between the producer and receiver at the time of calling was determined by calculating the difference in depth between the two tagged animals at time of call production. To investigate whether vertical distance could serve as a proxy for absolute distance between two calling individuals, absolute distance was assessed for a subset of the calls. Distances were calculated from the travel time of detected calls (time the call travelled from the producer to the recorder tag). Because the time-synchronisation had to be very precise for this method, only calls from sections in which both individuals vocalised could be included in our analysis. Consequently, absolute distance measurements were only possible for one of the three tag pairs, due to the lack of a second hydrophone or not enough simultaneous calling or clicking for the other two pairs. Absolute distance measures for calls were verified by calculating absolute distance from temporally close clicks, which have a clear onset time and are therefore less prone to measurement error. All acoustic analyses were performed in Matlab R2017a (the Math Works Inc., USA) using the DTAG toolbox (Johnson 2005).

### *Statistics*

The effect of call characteristics at the producer on call detectability at the receiver was modelled using a generalised linear model (GLM) with a binomial distribution. The full model included 1) call duration, 2) peak frequency, 3) ambient noise level at the receiver, 4) call PSD at peak frequency at the producer and 5) tag pair as explanatory variables, as well as all possible two-way interaction terms. It did not include distance, as analysis of vertical vs. absolute distance showed that these two were not correlated and absolute distance could only be measured for the periods when calls were produced and detected on both tags. The difference between absolute and vertical distance, where it could be assessed, ranged from 16 to 418 m.

The relation between all three call characteristics that could be influenced by the producer (duration, peak frequency, call PSD at peak frequency) and two environmental variables (total ambient noise level at the producer and producer depth) was modelled using three separate linear models with each of the call characteristics as the response variable and the other call and environmental variables as explanatory variables, including all two-way interactions between the two explanatory call characteristics and depth. Peak frequency and depth were log-transformed to maintain the assumption of normality and modelled using a Gaussian distribution. Call duration was modelled using a Gamma distribution. There was one outlier for peak frequency, which was excluded from the model.

Model selection was performed using dredging, based on lowest Akaike Information Criterion corrected for small sample sizes (AICc). All models that had AICcs within 2.0 points of the model with the lowest AICc were ranked based on lowest degrees of freedom (df). For all these models, $R^2$ values were calculated using the package piecewiseSEM (Lefcheck et al., 2018). The best model was the one with the lowest df. All statistics were performed in Rstudio 3.3.2. (R Core Team 2015).

## Results

### *Theoretical call propagation and detection*

Our model revealed that the detection range for call components with a peak frequency at 1 kHz was 230-1279 m. Higher frequency call components at 5 and 20 kHz would be detectable at minimally 1786 and 1414 m, respectively, with maximum ranges reaching beyond the modelled 2000 m. The maximum separation distance we could assess empirically (433 m) fell within these modelled ranges. Examination of click-based absolute distance measures that were close in time to calls showed an overestimation of 41-54 m for the call-based distance measures. The empirically assessed maximum separation distance therefore became ~385 m.

The counterintuitive increase in detection range at higher frequencies was due to the markedly decreased ambient noise levels at 5 and 20 kHz compared to 1 kHz. Tones at 1 and 5 kHz showed no significant difference in propagation distance due to limited attenuation ($\alpha$) at low frequencies (Fig. 3). Comparison of modelled ambient noise levels against the pilot whale audiogram indicated that all ambient noise levels were above unmasked hearing thresholds, hence detection was ambient noise limited. Ambient 1/3-octave band PSD levels at the receiver tag decreased with increasing frequency from 68-80 to 45-57 dB re 1 $\mu Pa^2$/Hz (25-75% quantiles) at 1 and 5 kHz, respectively, and were estimated by a Wenz-curve noise level at 50 dB re 1 $\mu Pa^2$/Hz at 20 kHz. Average ambient noise levels for 1 and 5 kHz (78 and 56 dB re 1 $\mu Pa^2$/Hz) were similar to reported ambient noise levels for sea state conditions at the time of measurement (sea state = 1-2 Beaufort; Wenz 1962). Since the critical ratios that were used increased with frequency, the net result was a similar average detection threshold for 5 and 20 kHz, and a much higher detection threshold for 1 kHz.

### *Empirical evidence for social call detectability*

We analysed social call detection probability in three pairs of tagged pilot whales. Recording duration ranged from 17-170 minutes, with a mean (s.d.) of 53 (27) calls per tag (Table 1). Individuals dove to a maximum depth of 401 m. The maximum depth difference between the tag pairs was 400 m. Absolute distance for the subset of calls where it could be estimated, ranged from 60-385 m. This was within the threshold detection range as predicted by the propagation model (1786 m), indicating that the tagged pairs remained within detection distance for at least part of the separation period. On average, 65% of calls produced by a tagged individual were detectable on the tag of the receiver (90% observer agreement). Calls that were detected on the receiver tag had a mean signal excess (eq. (2)) of 31 dB (range: 8-65 dB; Fig. S1). Detected calls had a lower peak frequency than undetected call (median 5 vs. 6 kHz) and were recorded at lower levels of ambient noise across the frequency range (Fig. 4).

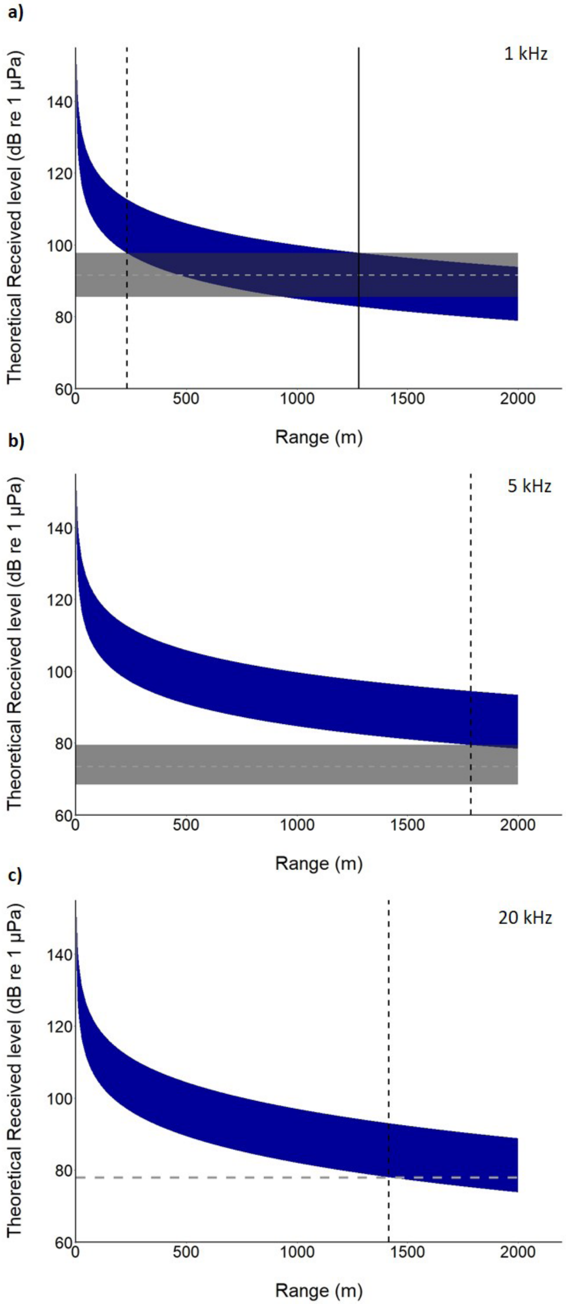

Figure 3. Sound propagation modelling for a pure tone (dark blue) of A) 1 kHz, B) 5 kHz, and C) 20 kHz with source level = 145-160 dB over a range of 2000 m. Median with 25 and 75 percentile detection thresholds (grey shaded area) and Wenz ambient noise level (grey dashed line) influenced signal detection at frequency-specific distances. Signals with a low source level dropped below the maximum detection threshold at some point for all frequencies (black dashed vertical lines). For 1 kHz signals, also signals with a high source level dropped below the detection threshold at 501 m (solid black line).

Table 1: Summary of tagged pair datasets. Record duration analysed was determined as a subset from temporal overlap between tagged-pair records.

| Tag ID | Record duration analysed (s) | Produced calls | Detected calls | Max dive depth in used data (m) | Deployment |
|---|---|---|---|---|---|
| 137a | 10223 | 70 | 25 | 288 | 17-5-‘09 14:52 |
| 137c | 10223 | 96 | 47 | 293 | 17-5-’09 15:52 |
| 138a | 1482 | 46 | 29 | 401 | 18-5-’09 12:17 |
| 138b | 1482 | 17 | 12 | 120 | 18-5-’09 13:19 |
| 158c | 1301 | 45 | 41 | 18 | 07-06-’10 17:52 |
| 158d | 1301 | 41 | 34 | 17 | 07-06-’10 17:55 |

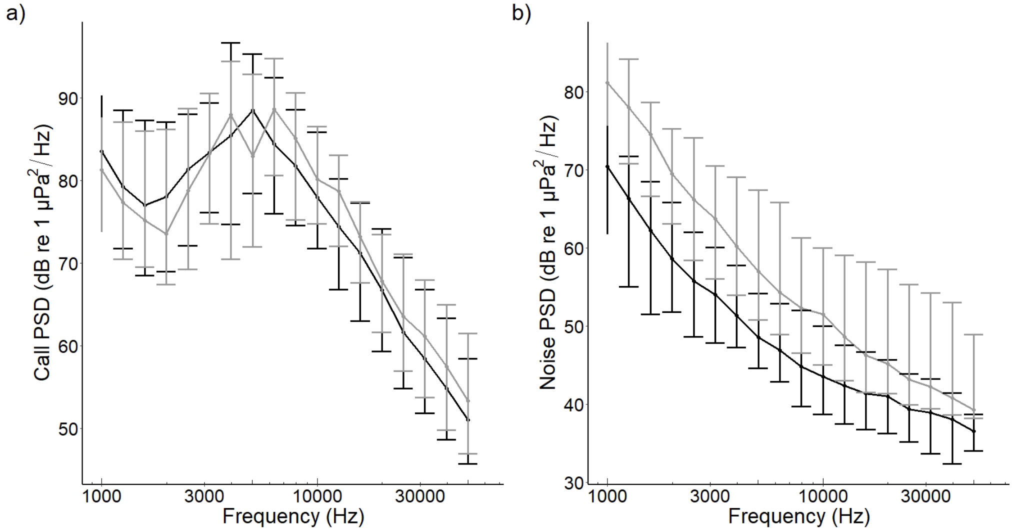

Figure 4: Average social call and ambient noise power spectral density of root-mean-square sound pressure (PSD) level for social calls and ambient noise, comparing conditions for detected (black, N = 183) and undetected (grey, N = 121) calls. Measures represent third octave band levels from 1-50 kHz of a) median call level at the producer and b) median ambient noise level at the receiver (corrected for flow noise). Error bars represent 25 and 75 percentiles.

### *Variation in call characteristics with context*

Call detectability in the empirical dataset was influenced by peak frequency, duration, call PSD level at the producer and ambient noise PSD level at the receiver ($R^2 = 0.48$, df = 13; Table S1). Detected calls were significantly longer than undetected calls (estimate = -1.24; $p<0.005$; Table 2) and had a slightly lower peak frequency ($p<0.005$, estimate = 0.000108). Detected calls of pair 158 were also produced at higher call PSD level than undetected calls ($p<0.05$; Fig. 5a-c). Furthermore, for detected calls, ambient noise PSD levels at the receiver side were significantly lower than for undetected calls, with a larger difference for pairs 138 and 158 than for pair 137 (Table 2; Fig. 5d).

Independent of detectability, call characteristics at the producer showed a number of interesting correlations, often dependent on environmental conditions. The best models

Table 2: GLM results testing how call detectability was affected by call and environmental characteristics.

| **Coefficients** | **Estimate** | **SE** | **Z** | **P** |
|---|---|---|---|---|
| Intercept | 0.15 | 2.00 | | |
| Pair | | | | |
| 138 | -6.00 | 3.45 | -1.74 | 0.08 |
| 158 | 8.26 | 5.15 | 1.61 | 0.11 |
| Peak frequency | 0.000108 | 0.0000371 | 2.99 | <0.005 |
| Noise at receiver | 0.0554 | 0.0187 | 2.96 | <0.005 |
| Call at producer | -0.0303 | 0.0196 | -1.55 | 0.12 |
| Duration | -1.24 | 0.415 | -2.99 | <0.005 |
| Duration:138 | -2.59 | 1.57 | -1.65 | 0.10 |
| Duration:158 | -4.61 | 2.09 | -2.20 | <0.05 |
| Noise at receiver:138 | 0.0962 | 0.0485 | 1.99 | <0.05 |
| Noise at receiver:158 | 0.0995 | 0.0493 | 2.02 | <0.05 |
| Call at producer:138 | -0.00269 | 0.0371 | -0.072 | 0.94 |
| Call at producer:158 | -0.171 | 0.0719 | -2.38 | <0.05 |

of the call characteristics showed several correlations: longer calls were louder and the relationship between call duration and call PSD changed with call peak frequency (interaction $p<0.05$; Fig. 6a, Table S1). Call characteristics were also influenced by depth of the producer: calls at depth were shorter and louder, especially higher frequency calls (Fig. 6b & c). Calls produced in higher ambient noise conditions were also louder and lower in peak frequency (Fig. 6d).

## Discussion

Our results demonstrate that social, deep-diving toothed whales can use social calls for acoustic relocation of close associates. Model propagation results showed that pilot whale social calls have the potential to be used for communication or as an acoustic beacon between vertically dispersed group members over 230-1786 m. Empirical data from three pairs of synchronously tagged animals confirmed our modelling results. 50% of produced calls were detected on receiver tags up to at least 385 m. Call characteristics influenced their propagation capacity. Modelled calls with a peak frequency of 1 kHz could be detected over shorter ranges than their counterparts at higher frequencies (5 or 20 kHz). This was caused by higher ambient noise levels at 1 kHz than at higher frequencies (propagation model results). Detected calls were also longer, had a higher power spectral density (PSD) level at the producer and were received in environments with lower ambient noise levels (empirical data). Plasticity of call production was indicated from differences in call characteristics under varying levels of ambient noise. In a noisier environment, calls were slightly louder and had a slightly lower peak frequency.

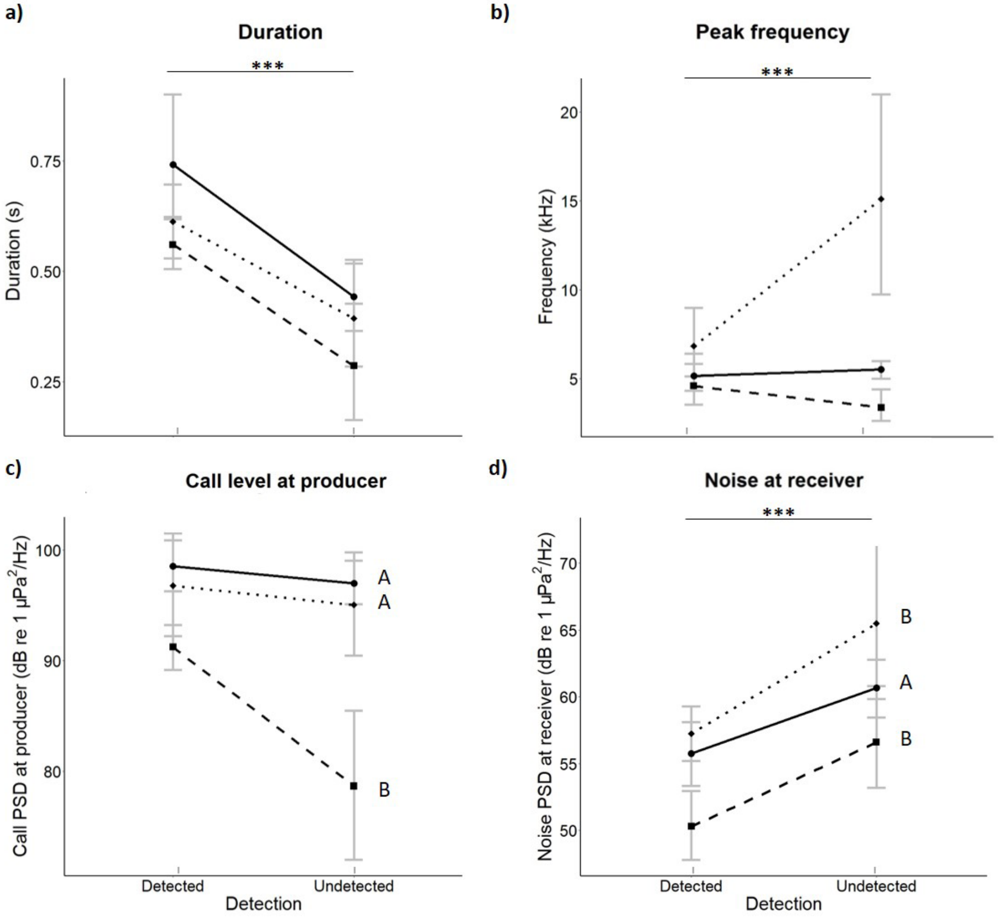

Figure 5: Call and environmental characteristics influencing social call detection. Detected and undetected calls were compared for a) call duration, b) call peak frequency, c) producer call power spectral density (PSD) at peak frequency, and d) ambient noise PSD at call peak frequency, at receiver. Comparisons are shown separately for each tagged pair (pair gm137: filled circle and solid line, Detected N = 72, Undetected N = 94; pair gm138: filled square and interrupted line, Detected N = 41, Undetected N = 22; pair gm158 = filled diamond and dotted line, Detected N = 75, Undetected N = 11). Error bars show bootstrapped 95% CI.  * indicates significance at * < 0.05, ** < 0.01 and *** < 0.0001, letters indicate pairs that showed equal trends.

### *Acoustic potential for group relocation*

The predicted large detection range for calls of 5-20 kHz (1414-1786 m) suggests that dispersed long-finned pilot whales maintain the potential to communicate during periods of vertical separation. These distances are at or exceeding pilot whale maximum dive depths recorded across populations (~600 m Baird et al. 2002; Sivle et al. 2012; Aoki et al. 2013; Visser et al. 2014; Aoki et al. 2017; Isojunno et al. 2017; Visser et al. 2017). Although the propagation model did not account for complexity in the transmis sion loss pattern when whales would also be horizontally dispersed, at short horizontal compared to vertical ranges these patterns are likely of minor influence to the overall

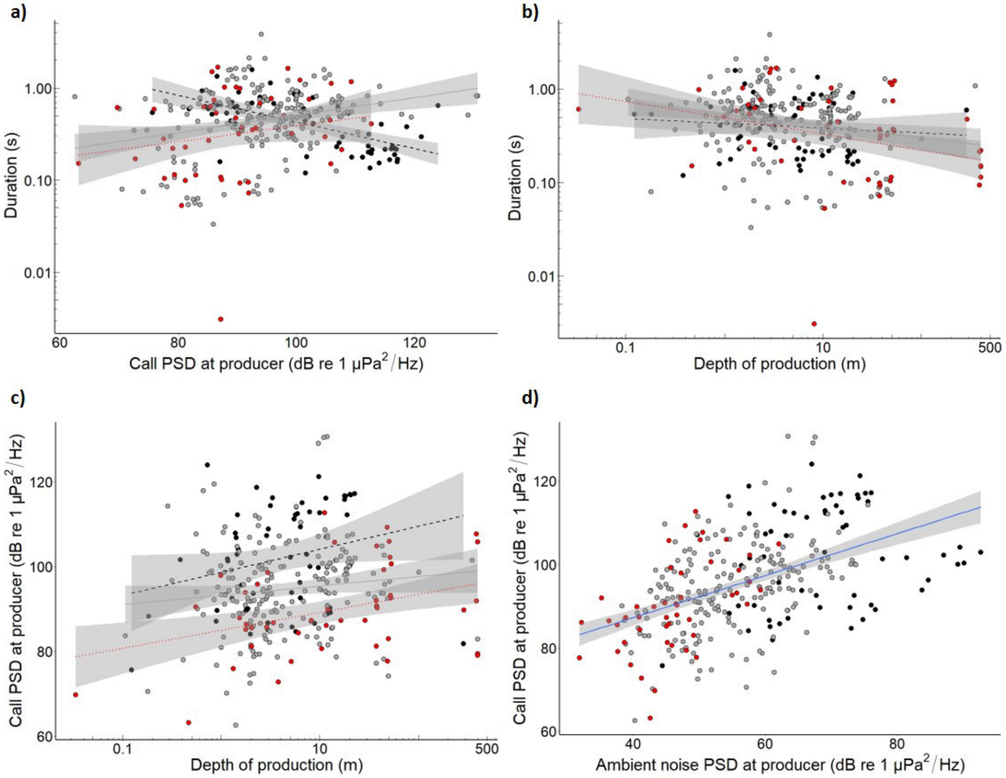

Figure 6. Call characteristics at the producer in relation to environmental features as a function of call peak frequency. The call peak frequency influenced the relation between a) producer call Power Spectral Density (PSD) at peak frequency and call duration, b) depth of call production and call duration, c) depth of production and call PSD and d) PSD of the ambient noise at the producer, at peak frequency and call PSD. Call peak frequency was divided into three subgroups for visualisation purposes: <2.5 kHz (black dots, black interrupted line, N = 61), 2.5-7.5 kHz (grey dots, grey solid line, N = 196), and >7.5 kHz (red dots, red dotted line, N = 58). Shaded areas represent 95% CI. Note that depth of production and duration are presented on a log$_{10}$ scale.

propagation distance (Ainslie 2010). Group members calling at the surface may serve as an acoustic beacon for listening group members that have been foraging at depth and aim to return to the group, similar to male frogs searching for chorusing conspecifics they can join (Bee 2007). These results complement previous findings, reporting context-dependent occurrence of calls in long-finned pilot whales with peaks in production of social calls at the start and end of foraging bouts of individuals (Visser et al. 2017). Thus, social calls were produced at or close to the surface while other group members might still have been foraging or returning to the surface. Empirical evidence for the communicative role of these calls, however, remains difficult, as it would require proof of vocally mediated shifts in upward swimming directions or turning angles in response to the location of a known producer.

As the detection of calls in this study was determined by human observers, we cannot exclude the possibility that pilot whales may have detected fewer or more calls than were detected by the observers. Since we excluded low-amplitude calls, the number of detected calls in this study is a conservative estimate. However, it is highly likely that all "detected" calls were also detected by the receiving whale. The maximum distance found for the subset of detected calls fell within the range predicted by the theoretical propagation model. Estimated call source levels from a subset of calls from one tag pair were consistent with the assumed range of source levels based on source levels reported in the literature. Furthermore, detected calls had a received level that was considerably higher than the detection threshold (mean signal excess: 30 dB; based on measured ambient noise levels and known critical ratios). Other mechanisms, such as comodulation masking release and directional masking release, might also contribute to higher rates of detected calls (Branstetter et al. 2008; Erbe et al. 2016). Nevertheless, it remains unkown at what signal excess level and with how much spectral information call detection translates to actual information transfer.

Even though it may appear obvious that social calls, often referred to as contact calls, serve in keeping contact with group members and relocating the group when separated (Ford 1989), actual proof is not abundant in any taxon and distinct call categories are far from clear (e.g. birds: Hamilton 1962; Marler 2004). However, several studies on terrestrial mammals reported the production of separation calls (Alberghina et al. 2013; Mumm et al. 2014). In a field study on chacma baboons (*Papio cynocephalus ursinus*) for example, so-called contact barks in adult females were induced by separation from the group at large or when separated from dependent infants (Rendall et al. 2000). A rare actual test of phonotactic potential of such calls in free-ranging capybaras (*Hydrochoerus hydrochaeris*) showed that playback of conspecific click calls interrupted the capybaras' behaviour and made them approach (Nogueira et al. (2012).

Odontocetes may be vocally active for other reasons than keeping the group together (Taruski 1979; Weilgart and Whitehead 1990; Zwamborn and Whitehead 2016) and do not need to have the intention to allow homing by sound for foraging group members. However, the temporal patterns of vocal activity are so that group cohesion can be mediated by phonotactic behaviour (Jensen et al. 2011; Marrero Pérez et al. 2017; Visser et al. 2017). Being more silent when in larger, more tightly spaced groups, and more vocal during foraging bouts when group members are repeatedly leaving and returning for deep foraging bouts also seems to be quite widespread among whales and dolphins (Tyack 2000; Visser et al. 2014) and supports the hypothesis that acoustic communication is used to maintain contact between group members.

### *How call characteristics influence detection*

Our finding that several call features such as duration, amplitude and frequency at the producer correlated with the detection probability at the receiver is in line with our knowledge about the effect of propagation and ambient noise on evolutionary shaping of acoustic signals (Ryan and Brenowitz 1985; Endler 1992; Brumm and Slabbekoorn 2005). As expected, longer and louder calls were better detectable. The empirical data further showed a statistically significant difference in peak frequency between detected and undetected calls, but this difference was so small that it was likely not biologically relevant. It could be because system noise in the higher frequencies limited our ability to detect high frequency calls. Propagation model results showed that calls of 1 kHz would actually be less detectable than higher frequency calls, due to high ambient noise levels at that frequency. These modelled detection thresholds suggested that calls of 5 and 20 kHz would be equally well detectable, with a possible increase in detection at 20 kHz when the increased hearing directionality with increasing frequencies is taken into account.

Interestingly, the pilot whale calls measured had substantial energy at frequencies below 5 kHz, which would be suboptimal for the range of call detection. However, the high ambient noise level at 1 kHz that caused the shorter detection range is an evolutionary recent phenomenon. At low ambient noise levels, calls of 1 kHz would likely have a larger detection range than calls at higher frequencies, due to a smaller loss from frequency-specific attenuation. There could also be trade-offs between optimal call features for propagation and constraints on production or limitations by depth. Indeed, we found that calls at depth were shorter, similar to results found for short-finned pilot whales (Jensen et al. 2011), but also louder, especially the high-frequency calls (Fig. 6). An alternative route, diminishing these potential trade-offs, is the evolution of more extreme call features or higher auditory sensitivity. Sperm whales, for example, are highly social deep divers, foraging at depths up to 1800 m. Their echolocation clicks are the most intense animal-borne signals known to date (Møhl et al. 2002).

### *Rising levels of ambient noise*

The potential importance of hearing calls from group members and the fine-tuning to the acoustic environment may make deep-diving odontocetes vulnerable to current changes in the oceans. Ambient noise levels have increased due to a wide variety of human activities, including container shipping, seismic exploration, pile driving and deep-sea mining (McDonald et al. 2006; Hildebrand 2009; Frisk 2012), though the trends in ambient noise may be site-specific (Miksis-Olds and Nichols 2016). Although masking issues can be relieved through differences in location between the anthropogenic source and the caller (directional masking release), or through the fluctuating amplitude in the ambient noise levels (co-modulation masking release; Brumm and Slabbekoorn 2005; Branstetter et al. 2008; Moore 2013), it is important to investigate what the implications will be when group members do get separated by increasing ambient noise levels.

Pilot whale social calls that were produced during higher levels of ambient noise in the current study had a higher PSD level and a lower peak frequency than calls produced during quieter periods (Fig. 6d). This indicates a level of plasticity that can be employed in natural ambient noise level fluctuations, e.g. through increased sea state, and could to some degree also be successful in response to a rise in anthropogenic ambient noise levels (cf. Foote et al. 2004; Miller 2006; Parks et al. 2011). However, a proper cost-benefit analysis is required to explore whether the extent and volitional control of such plasticity is sufficient to prevent negative consequences to the individual or population

(Southall et al. 2007; Holt et al. 2015).

### Conclusion

We have shown that deep-water foraging pilot whales have the capacity to communicate with their group members at the surface, using frequency-modulated tonal calls. This potential was found both through propagation modelling and empirical data of produced and received calls on simultaneously tagged group members. The variation in social call characteristics such as PSD level and duration influence the communication range, representing call-type plasticity which might be utilized to overcome masking in situations of increased ambient noise levels. However, as call structure also changes with depth, there might be physical restrictions to this variability. It is therefore of great importance to investigate whether anthropogenic activity at sea will lead to impaired communication between vertically dispersed group members of this highly social species.

## Acknowledgments

The authors are grateful for the effort put in by all members of the 3S-cruise in 2009 and 2010 for collecting the data. Paul Wensveen kindly made his script for click extraction available to us. Michael Ainslie, Ozkan Sertlek, Mark Prior, Mark Johnson, Coen Elemans and Philip Bijl aided with the acoustic analyses and with constructing several versions of the propagation model. Data collection for this project was funded by Office of Naval Research awards [N00014-08-1-0984, N00014-10-1-0355, N00014-15-1-2341], the Dutch Royal Navy, and the Norwegian Navy (FFI).

# Appendix

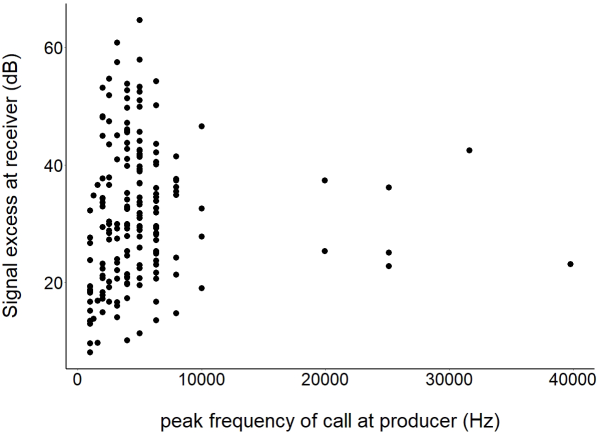

Figure S1: Signal excess (the level of the signal above the detection threshold) of calls that were detected on the tag of the receiving whale. These values give an indication of the audibility of the calls for the receiving whale.

Table S1: GLM model selection. Best model selection was based on lowest Akaike Information Criterion corrected for small sample size (AICc), after which the final model was selected from all models with a non-significant difference from the lowest AICc (ΔAICc<2). The final model (bold) was then selected to be the model with the lowest degrees of freedom (df). Interaction terms are indicated with ":" between variables. All variables were measured on the tag of the producer, except ambient noise levels (Noise Receiver).

| **Pair** | **Duration** | **Peak frequency** | **Noise Receiver** | **Call producer** | **Pair:Duration** | **Pair:Frequency** | **Pair:Noise Receiver** | **Pair:Call producer** | **Duration:Frequency** | **Duration:Noise Receiver** | **Duration:Call Producer** | **Noise Receiver:Call Producer** | **df** | **R$^2$** | **AICc** | **ΔAICc** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| x | x | x | x | x | x |  | x | x | x |  |  |  | 14 |  | 311.1 | 0 |
| x | x | x | x | x | x |  | x | x |  |  |  |  | 13 | 0.48 | 312.3 | 1.20 |
| x | x | x | x | x | x |  | x | x | x | x |  |  | 15 |  | 312.9 | 1.82 |
| x | x | x | x | x | x |  | x | x | x |  | x |  | 15 |  | 313.0 | 1.94 |

Table S2: LM results investigating how duration, call SPSD at the producer, and peak frequency were correlated with several call variables and depth. Duration, peak frequency and depth were log transformed to maintain normality assumption. Interaction terms are indicated with ":" between variables. Only the variables retained in the final models are included.

| **Model** | **Coefficients** | **Estimate** | **SE** | **T** | **P** | **R$^2$** | **AICc** |
|---|---|---|---|---|---|---|---|
| *Duration* | Intercept | 19.59 | 4.11 | | | 0.21 | 150.4 |
| | log(Depth) | 1.15 | 0.27 | 4.25 | <0.0001 | | |
| | Call SPSD at prod | -0.24 | 0.045 | -5.32 | <0.0001 | | |
| | log(Frequency) | -2.48 | 0.49 | -5.02 | <0.0001 | | |
| | Call:log(freq) | 0.030 | 0.0055 | 5.45 | <0.0001 | | |
| | log(Depth):log(Freq) | -0.15 | 0.031 | -4.85 | <0.0001 | | |
| *Call SPSD at prod* | Intercept | 62.40 | 13.74 | | | 0.43 | 2297.8 |
| | log(Depth) | -9.99 | 3.58 | -2.79 | <0.01 | | |
| | log(Duration) | -38.49 | 9.21 | -4.18 | <0.0001 | | |
| | log(Frequency) | 1.31 | 1.35 | 0.98 | 0.33 | | |
| | Noise at prod | 0.38 | 0.069 | 5.57 | <0.0001 | | |
| | Tag | | | | | | |
| | 137c | 1.70 | 1.53 | 1.11 | 0.27 | | |
| | 138a | -0.88 | 1.79 | -0.49 | 0.62 | | |
| | 138b | 11.33 | 2.59 | 4.37 | <0.0001 | | |
| | 158c | -2.77 | 1.83 | -1.51 | 0.13 | | |
| | 158d | -8.09 | 1.89 | -4.29 | <0.0001 | | |
| | log(Depth):log(Freq) | 1.32 | 0.41 | 3.21 | <0.005 | | |
| | log(Dur):log(Freq) | 4.73 | 1.09 | 4.34 | <0.0001 | | |
| *Frequency* | Intercept | 10.82 | 0.49 | | | 0.57 | 466.3 |
| | log(Depth) | -0.55 | 0.15 | -3.73 | <0.0005 | | |
| | Call SPSD at prod | -0.00011 | 0.0052 | -0.021 | 0.98 | | |
| | log(Duration) | -0.70 | 0.34 | -2.07 | <0.05 | | |
| | Noise at prod | -0.042 | 0.0032 | -12.99 | <0.0001 | | |
| | Tag | | | | | | |
| | 137c | -0.063 | 0.087 | -0.73 | 0.47 | | |
| | 138a | -0.11 | 0.10 | -1.09 | 0.28 | | |
| | 138b | 0.21 | 0.15 | 1.42 | 0.16 | | |
| | 158c | -0.35 | 0.10 | -3.57 | <0.0005 | | |
| | 158d | -0.54 | 0.10 | -5.20 | <0.0001 | | |
| | log(Depth):log(Dur) | -0.072 | 0.024 | -2.93 | <0.005 | | |
| | Call at prod:log(Dur) | 0.0097 | 0.0038 | 2.56 | <0.05 | | |
| | log(Depth):Call at prod | 0.0063 | 0.0015 | 4.27 | <0.0001 | | |

# *How noise affects prey*

# Effects of impulsive, low frequency anthropogenic noise on pelagic fish in the North Sea

Annebelle C. M. Kok, Lisa Bruil, Benoit Berges, Serdar Sakinan, Elisabeth Debusschere, Jan Reubens, Dick de Haan, Alain Norro, and Hans Slabbekoorn

## Abstract

Anthropogenic noise in the oceans is disturbing marine life. Pelagic fish are a group of species that are also likely to be affected by noise, but have received little attention because of the difficulties of studying them. In wind farms, pelagic fish abundance could be higher than in other areas, due to fisheries restrictions. At the same time, the wind farm construction involves a lot of anthropogenic noise, likely disturbing marine life. Here, we investigate whether bottom-moored echosounders are a suitable tool for studying the effects of anthropogenic sound on pelagic fish in wind farms and explore the types of responses to anthropogenic noise exhibited by pelagic fish. Two bottom-moored echosounders were placed in three different wind farms along the Dutch and Belgian coast, recording presence and behaviour during exposure to the passing by of a full-scale airgun array from an experimental seismic survey and pile driving activity in an adjacent wind farm construction site. The bottom-moored echosounders were successful in detecting variation in the behaviour of pelagic fish residing at the wind farms. Patterns of behaviour and detection of fish schools were significantly different during sound exposure compared to pre-exposure, with fewer, more cohesive schools during the seismic survey and more cohesive schools swimming higher in the water column during pile driving. The types and magnitudes of responses, however, were also observed at the control site. While this stresses the need for thorough replication when investigating responses of pelagic fish to sound exposure, our results indicate that both sound from a seismic survey as well as from pile driving could have the potential to disturb pelagic fish school behaviour.

## Introduction

Many aquatic animals change their behaviour in response to increased ambient noise levels. Effects of sound on behaviour range from local changes in water column use (Hawkins et al. 2014b; Neo et al. 2014), to horizontal avoidance of noisy areas (e.g. Carstensen et al. 2006; Kok et al. 2018) and may include changes in mate choice, foraging behaviour, and anti-predator responses (Shafiei Sabet et al. 2015; Simpson et al. 2015; de Jong et al. 2018; Chapter 6). Increased noise levels have been found to affect all trophic levels, from invertebrates (Hubert et al. 2018) to top predators, such as marine mammals (Southall et al. 2016). Specifically, changes in predator-prey interactions have a strong potential to translate to effects on the ecosystem as a whole (Kunc et al. 2016).

Predator-prey interactions are characterized by specific behaviour of the predator – hunting – and responding behaviour of the prey – hiding, escape and defence. Prey defence tactics in the ocean, where hiding in vegetation or under rocks is no option, often involve aggregation into groups. For example, prey species in deep-scattering layers have been shown to cluster to improve protection and reduce risk of attack of individual animals by being in a group (Benoit-Bird et al. 2017). Furthermore, pelagic animals change their location in the water column in response to predators, often by moving down (Hawkins et al. 2014b; Neo et al. 2014; Rieucau et al. 2014). At the same time, predators aim to maximize their success by targeting high-density prey areas and adapting hunting strategies to prey behaviour (Charnov 1978; Au et al. 2013). For example, marine mammal predators that forage on clusters of animals in deep scattering layers often target their prey at night, when the deep scattering layer migrates closer to the surface (Au et al. 2013; Giorli et al. 2016). Therefore, environmentally induced changes in prey defence behaviour are likely to also alter hunting strategies of their predators.

Acoustic disturbance related changes in prey behaviour could have important consequences for predators. It could be beneficial, if prey become less vigilant and are consequently more easily caught (Simpson et al. 2015). Or it could be disadvantageous, for instance if prey become more cohesive and move deeper down the water column, and therefore become more difficult to catch (Voellmy et al. 2014b). In fact, how prey respond to increased noise levels could even determine the response of predators to sound. Cuvier's beaked whales (*Ziphius cavirostris*), for instance, stayed in an area that was frequently disturbed by military sonar exercises, but also contained high prey densities, and did not alter their hunting grounds to an adjacent canyon that was less disturbed by sonar but also contained less prey (Southall et al. 2019). So, understanding how prey will respond to sound might also provide information on the potential effect of sound on their predators.

Many animals from the bottom and middle trophic levels are pelagic, such as zooplankton and schooling fish. However, the effects of sound on pelagic animals have hardly been studied. One benchmark study by Hawkins et al. (2014b) indicated changes in cohesion and vertical displacement of pelagic fish and zooplankton when exposed to an artificial, intermittent sound. Additionally, a case study in which zooplankton was experimentally exposed to a seismic survey showed increased mortality compared to the period before the survey (McCauley et al. 2017). Apart from these studies, that require follow-up and replication, a few studies reported changes in fisheries catch rates during and after a noisy human activity, such as a seismic survey (e.g. Skalski et al. 1992; Parry and Gason 2006; Løkkeborg et al. 2012). Consequently, we still lack sufficient insight into changes in spatial behaviour of pelagic species.

Offshore wind farms provide an interesting opportunity to study the pelagic community as well as the potential effects of anthropogenic noise. More and more offshore wind farms are being constructed to exploit the renewable source of wind energy, with supposedly little or even positive environmental impact (Lindeboom et al. 2011; Ashley et al. 2014; Raoux et al. 2017). In the pre-construction and construction phase, however, seismic surveys and pile driving activities typically cause considerable acoustic disturbance in the area. Subsequently, in the exploitation phase, a moderate, low-frequency noise from the wind-driven rotor blades remains, while scour beds and the set of piles and control stations typically introduce a rocky reef at places that were dominated by sandy bottom. Consequently, over time, a different and more diverse benthic community develops (Lindeboom et al. 2011; Ashley et al. 2014; Raoux et al. 2017), which may also affect the local pelagic community. Therefore, changes in pelagic fish behaviour due to anthropogenic noise can only be discovered with long-term measurements that control for the natural development of the community.

A way to study long-term presence and behaviour of pelagic fauna is through the use of bottom-moored echosounders. An echosounder system operates by transmitting ultrasonic pulses, which are backscattered by objects and detected by a receiver (Lurton 2002). Because of their non-invasiveness and spatial resolution, echosounders are widely used to identify individual species and to observe fish school behaviour in the water column. They are also used to monitor changes in school cohesion and swimming depth (Gerlotto et al. 2004; Weber et al. 2009; Guillard et al. 2010; Hawkins et al. 2014b; Fraser et al. 2018), which are among the expected responses to seismic surveys and pile-driving (Lawson et al. 2001; Colbo et al. 2014; Benoit-Bird et al. 2017).

Here, we explored the effects of two types of anthropogenic sound events – an experimental seismic survey and nearby pile driving – on the spatial behaviour of pelagic fish in the North Sea. We investigated whether bottom-moored echosounders are a suitable tool for studying the effects of anthropogenic sound on pelagic fish, and whether there are longer-term (days) changes in pelagic biomass or behaviour that can be correlated to seismic survey or pile driving sounds. To be able to observe spatial behaviour of the pelagic fish layer, we deployed a pair of bottom-moored echosounders that recorded the entire water column over the course of a month, replicated at three locations in three subsequent periods. By placing an echosounder both inside and outside the wind farms, we aimed to sample the variation in pelagic fish related to these ecologically distinct locations with either rocky or sandy bottom. We expected anthropogenic noise exposure to be correlated with deeper swimming pelagic biomass, as well as fewer, more cohesive, and deeper swimming fish schools.

## Materials and methods

### Echosounders

Two Acoustic Zooplankton Fish Profilers (AZFPs, ASL Environmental Sciences, Canada) were deployed consecutively at three wind farms. Both AZFP echosounder sets emitted four frequencies, of which three were shared (due to the availability of this equipment at ASL): 125, 200 and 455 kHz. The first and second AZFP also transmitted at 38 and 769 kHz, respectively. The first AZFP was always placed inside the wind farm, whereas the second AZFP was always placed outside the wind farm. Both AZFPs were moored on a frame at the seafloor, at an average depth of 32 m, and had a vertical upward beam (Table 1). The AZFPs recorded 25 consecutive days per location with a ping rate of 1 Hz (sound pulses emitted by the echosounder). Data was extracted after retrieval of the AZFPs.

Table 1: Description of deployment locations, exposure type, and period, placement, and depth of deployment.

| **Location** | **Exposure** | **Deployment (2018)** | **Recordings (2018)** | **Location AZFP** | **Depth of deployment (m)** |
|---|---|---|---|---|---|
| Wind farm SEISMIC | Seismic survey | 10/7 – 20/8 | 15/7 – 11/8 | Inside | 37.5 |
| | | | | Outside | 38 |
| Wind farm PILE | Pile driving | 20/8 – 22/9 | 22/8 – 22/9 | Inside | 24 |
| | | | | Outside | 25 |
| Wind farm CONTROL | Control | 13/11 – 12/12 | 18/11 – 18/12 | Inside | 32.7 |
| | | | | Outside | 33.1 |

### Study locations

The AZFPs were placed at two wind farms in the Belgian and one wind farm in the Dutch North Sea: 1) Belwind – an offshore wind farm situated on Bligh bank, 40 km from the Belgian coast, 2) C-Power – an offshore wind farm situated at Thornton bank, 27 km off the Belgian coast and 3) Gemini, located 85 km from the Dutch coast, north of Schiermonnikoog (Fig.1). Belwind (wind farm SEISMIC) was exposed to an experimental seismic survey over a period of four days (Fig. 2; PCAD4Cod project, Slabbekoorn et al. 2019). C-Power (wind farm PILE) was exposed to pile driving from a nearby wind farm for 12 separate days during the AZFP deployment period for the construction of the offshore wind farm Norther. Gemini (wind farm CONTROL) was not exposed to any particular anthropogenic activity, other than shipping noise from local maintenance traffic and a nearby shipping lane and functioned as the control.

To investigate differences between the pelagic fish inside and outside the wind farm, at all three locations, one AZFP was placed inside the farm, 150 m from the centre of the wind farm, while the second AZFP was placed 700 m from the edge of the wind farm. AZFPs were always placed at equal distance to the anthropogenic noise disturbance, such that noise levels would not differ between the AZFP inside and outside the wind farm. The AZFPs were placed consecutively in the three wind farms: first at wind farm SEISMIC, next at wind farm PILE and finally at wind farm CONTROL (Table 1).

Water temperature, wave height and tide records were taken from the Dutch Ministry of Infrastructure and the Environment (waternet.nl, Rijkswaterstaat) from measuring stations close to the wind farms (see Table S1 for locations). Water temperatures were quite constant per wind farm: they varied between 16.8 – 18.7 °C at wind farm SEIS-

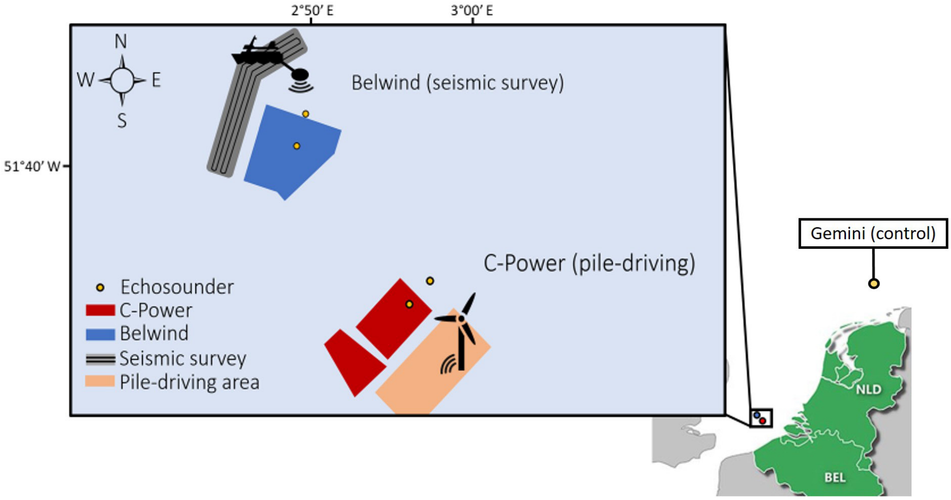

Figure 1: Schematic overview of the placement of the AZFP echosounders (yellow dots in inset) at the two wind farms in the Belgian North Sea. Note the roughly equal distance of the AZFPs to the track of the seismic survey (Belwind) and to the pile driving area (C-Power). At Gemini, there were no periods of impulsive anthropogenic noise during these measurements in the Dutch North Sea.

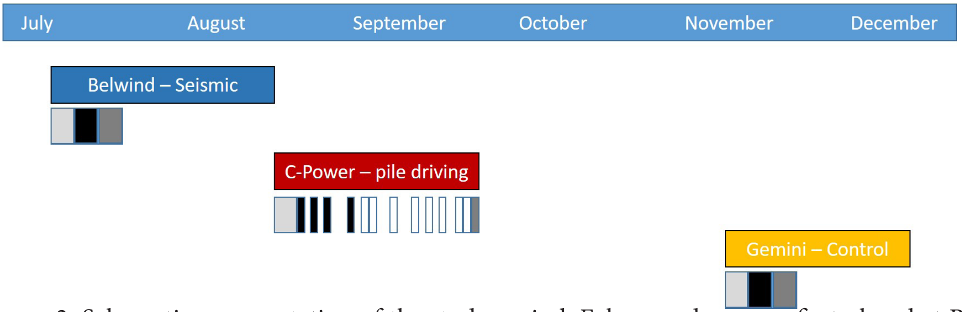

Figure 2: Schematic representation of the study period. Echosounders were first placed at Belwind (wind farm SEISMIC), next at C-Power (wind farm PILE) and finally at Gemini (wind farm CONTROL). Pelagic biomass was measured for the entire recording period (coloured named boxes). Fish schools were measured for four days before the exposure (light grey boxes), four days during the exposure (black boxes), and four days after the exposure (dark grey box). At C-Power pile driving exposure took place on 12 separate days, the first four of which were taken as exposure period (black boxes: measured exposure days; white boxes: not measured exposure days).

MIC, 17.3 – 19.2 °C at wind farm PILE, and 9.0 – 10.8 °C at wind farm CONTROL. Distance between the inside and outside frames ranged from 2.3-3.0 km (Table S2). The echosounder locations inside wind farm SEISMIC and wind farm PILE were 15.52 km apart, while the echosounder location inside wind farm CONTROL was 333 km (in a straight line) from both locations inside wind farms SEISMIC and PILE.

### Soundscape

The wind farms differed in the soundscape at the time of measurement. Sound measurements at wind farm SEISMIC were taken with a moored hydrophone (AMAR, M36, rented from JASCO) at 22 m depth inside the wind farm, anchored with a 60 kg rock. Ambient sound levels fluctuated with time but were on average 95-110 dB re 1 µPa$^2$/Hz at 10-500 Hz at wind farm SEISMIC and 56-87 dB re 1 µPa$^2$/Hz at 10-500 Hz at wind farm PILE. Ambient sound levels at wind farm CONTROL were not measured during the study but have been reported to range from 80-100 dB re 1 µPa$^2$/Hz at 10-500 Hz on average in 2013 (Lucke 2015).

### Sound exposure

A full-scale airgun array was used for an experimental seismic survey at wind farm SEISMIC from 21-24 July 2018 (Fig. 2). Sound levels at the echosounder ranged from 123 to 195 dB re 1 µPa $SPL_p$. The survey used 36 airguns (G-Gun II Sercel, 50% operating at a time) with a total volume of 5900 m3 (carried out by CGG, Norway, with the MV Geo Caribbean). The airgun arrays were towed 204 m behind the vessel, at a depth of 6 m below the surface. The survey involved 19 shooting lines with an average length of 22 km, except for the first line (30 km). Closest approach was 2.1 km from the wind farm. The air guns generated a sound pulse every 10 s, while the vessel maintained an average speed of 2.2 m/s. For the first line a soft-start procedure of 20-40 minutes was used.

Pile-driving was carried out next to wind farm PILE at a new wind farm site (51° 32’ N, 3° 2’ E) during the construction of additional wind turbines in an adjacent plot. Sound levels for one pile driving period were on average 172 dB re 1 µPa $SPL_p$ at both AZFPs. A total of 20 turbines were built during the period from 6 August to 25 September 2018, with pile-driving being conducted on twelve days within this period, all separated by one or more days of no activity. Of the twelve pile-driving days, the first four days were used for the behavioural analysis (Fig. 2). On those four days, all the pile-driving was carried out at daytime. The average pile-driving duration on these days was 148 minutes (range 100-180 minutes).

### Observation protocol

To investigate changes in pelagic fish abundance and behaviour between exposure and control conditions, we analysed biomass and school characteristics form the echosounder data. Total biomass was calculated per 1 m depth and 10 min bins for the entire survey period. The exposure period was the entire duration of sound exposure per site, with a control period of sham exposure for wind farm CONTROL (Table S3). All data points that did not fall in the exposure period were considered to be baseline. The presence of fish schools, as well as their distribution and size were measured during 4 day-periods before, during and after the exposure. The 4-day period was selected based on the duration of the seismic survey exposure (Table S3).

For wind farm SEISMIC, the BEFORE, DURING and AFTER period consisted of four consecutive days before, during, and after the seismic survey. Since the days of pile driving sound exposure at wind farm PILE were not consecutive, the DURING days were the first four days that contained pile driving after the start of deployment (August 26, 28, 30 and September 3). Even though pile driving only took place during part of those days, the entire days were sampled as DURING, to maintain similarity in measurement set-up with the other two wind farms. The BEFORE period of wind farm PILE started on the first day of deployment, after an eight-day silent period without pile driving in the area. The AFTER period started on the first day after an exposure period. Piling activity stopped one day before retrieval of the AZFPs, so the AFTER period lasted only one day. There was no period of four days without piling earlier in the deployment. For wind farm CONTROL, the first twelve days of deployment were arbitrarily selected as a control BEFORE, DURING, and AFTER period.

### *Data analysis*

All data analyses were performed using Echoview 9 (Echoview Inc.). The raw data were pre-processed to filter out noise and facilitate school detection (Fig. S1; *sensu* Trygonis et al. 2009). First, a maximum-strength echogram was calculated from all the measured frequencies, by taking the maximum echo strength from all frequencies per pixel. The optimal frequency for detection differs between species. By taking the maximum echo strength for all frequencies, we made sure that species type did not affect detection probability. Low-signal detections were removed from the maximum-strength echogram by implementing a -63-dB echo strength threshold. This procedure avoids the false detection of pelagic fish due to reflections that are too minimal to be fish.

To further remove noise in the data from non-biotic particles, we applied an erosion-dilation procedure (Haralick et al. 1987; Reid and Simmonds 1993). This procedure detects clusters of pixels with high echo strength, thereby favouring larger detected objects such as single, but relatively large fish or fish schools of small or large fish. The mask – i.e. a 'pattern' of detected and undetected pixels – was created by applying these procedures on the maximum-strength echogram, which was then put over the raw data of 125 kHz. Data for this frequency were present at both AZFPs, making it possible to compare measurements. Data with the mask were filtered with a threshold of -70 dB. Finally, echoes that were close to the surface or bottom were regarded as noise (i.e. waves, sediment particles) and were excluded from the data.

### Fish school detection

Fish schools were detected automatically using a built-in school detection function of Echoview (detection settings, Table S4). Detection settings were based on a comparison of automatically detected schools with manually detected schools in a subsample of the data. After automatic detection, all detected schools were checked manually to correct for false positives and false negatives by the algorithm. Schools were defined in two ways: 1) at least three separate traces of potential fish reflections, present in the same ping (sound pulse from the echosounder), with a maximum vertical distance of 1 m; 2) an area with increased echo strength of at least 1 m high during at least one ping (Fig. 3). Both school types had to be visible for at least 3 pings before being considered a school.

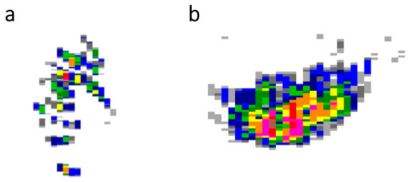

Figure 3: Different forms of a fish school in an echogram. (a) Separate traces with increased echo strength. (b) An area of increased echo strength without any evident separate traces. Colours indicate echo strength intensity, with warmer colours (e.g. orange, pink, and red) being a higher echo strength.

### Pelagic biomass measurements

Biomass was calculated as the Nautical Area Scattering Coefficient (the integrated scattering strength of a bin, NASC) for bins of 1 m depth by 10 minutes. NASC is defined as:

$$NASC = 4\pi Nm^2 10^{\frac{Sv}{10}} T \qquad (1)$$

Where *NASC* = Nautical Area Scattering Coefficient in $m^2/nm^2$, *4π* converts backscattering cross-section to scattering cross-section, *Nm* = a nautical mile in m (1852 m/nm), *Sv* = mean volume backscattering strength of the bin being integrated in dB re 1 $m^2/m^3$ and *T* = mean thickness of the bin being integrated.

For the biomass data of the entire survey period, the centre of gravity (i.e. mean depth of the biomass in the water column, henceforth described as biomass depth) was calculated per 10 min bin. The centre of gravity was taken as:

$$Centre\ of\ gravity = \frac{\sum momentum}{\sum NASC} \qquad (2)$$

With

$$momentum = NASC * D \tag{3}$$

Where *momentum* is in $m^3/nm^2$ and $D$ = distance from the AZFP in m. Since the biomass depth at a certain time point depended on the depth of the previous time point (temporal autocorrelation), the data was resampled to one 10-min bin every 3 hours.

### Fish school measurements

We took behavioural measurements from the detected schools based on reported responses of fish to sounds of seismic surveys and pile driving (Fewtrell and McCauley 2012; Hawkins, Roberts, et al. 2014). Reported responses included increased swimming depth and increased school cohesion. Swimming depth was measured as the mean distance of the school from the AZFP (which was always at the sea floor) in m. School cohesion was measured by the mean volume backscattering strength of the school (Sv) in dB re 1 $m^2/m^3$. An increase in the backscattering strength of the school equals an increase in the school density and thus indicates a smaller distance between individuals, i.e. a higher school cohesion.

Besides taking behavioural measurements per school, we measured fish presence by counting the number of schools present per hour, as well as the total biomass of these schools per hour (schooling fish abundance) in NASC calculated per school-'region per cell'-bin (PRC_NASC, Eq. (1)). The school was divided up in bins of 1 m by 10 min, and only the area of the bin covered by the school was taken. This way, larger schools would not be overweighed in the data. These values were then summed over each hour.

### Statistical analysis

We investigated whether sound exposure influenced biomass depth, as well as school presence, schooling fish abundance, school swimming depth, and school cohesion. All statistical analyses were performed using RStudio (version 3.5.1). Models were constructed using the biomass depth, school presence, schooling fish abundance, school depth and school cohesion as response variables and location (inside or outside the wind farm), wave height and tide (except in the model for school presence) as common explanatory variables. The model for biomass depth further included treatment (exposure or baseline), total biomass in the water column, and temperature as explanatory variables, as well as an interaction between treatment and location.

The models concerning school variables also included period (before, during, or after sound exposure) and an interaction between period and location. As temperature correlated strongly with period, we left temperature out of the school data models. The models for school depth and school cohesion further included the vertical spread of the school as explanatory variable, since that could influence these response variables.

Final models were selected using dredging (*MuMIn* package). After dredging, variable estimates were calculated by bootstrapping (10,000x). If estimates of the explanatory variables did not cross zero in the 95% confidence interval (CI), explanatory variables were considered to be of significant influence on the response variable.

Separate models were created per wind farm to account for the high variability between wind farms in time of year and location. All models used were either linear or generalised linear models (Table S5). We found the optimal distribution by checking model diagnostics (e.g. the QQ-plot of the model) and by testing for models with higher log-likelihood scores using lrtest (*lmtest* package). School presence was evaluated in two ways: first, by applying a rotation test (*tagtools* package; DeRuiter and Solow 2008) that examined whether the pattern of school detection was similar during exposure and before and after periods. This rotation test was applied on the entire 12 days of wind farm SEISMIC and CONTROL but was applied on the exposure days of wind farm PILE separately, to account for the discontinuity of the exposure periods. Second, we investigated if the number of schools per hour changed during exposure, using a Hurdle model that consisted of two parts. The first part separately modelled the chance that a school is present by treating all data points larger than zero as 1 (binomial distribution). The second part of the model ignored all data points that are zero and only modelled the number of schools that are present (negative binomial distribution). This part could then tell if the number of schools present was explained by the explanatory variables.

## Results

### *General patterns*

There was a distinct diurnal pattern of fish schooling during daylight and a layer of scattered individual fish in the water column at night with a clear transition between the two states at dawn and dusk in both wind farm PILE and wind farm CONTROL but not in wind farm SEISMIC (dusk; Fig. 4). Because the fish did not school at night-time, we decided to exclude the data between dusk and dawn from the analyses for both wind farm PILE and wind farm CONTROL (roughly 19:00 – 04:00h for wind farm PILE and 16:30 – 06:20h for wind farm CONTROL). At wind farm SEISMIC, no such pattern was visible, so schools were measured during day and night. Weather conditions varied considerably over the deployment periods, and calm to rough sea surface conditions were found for all wind farms, with decreased detection possibility of fish schools during rough sea states. Wave height could reach up to ~2.5 m at all wind farms.

Fish schools were found both inside and outside the wind farm at all three wind farms. There were a median of 2 schools per hour at wind farm SEISMIC (N = 573, range = 0-53 schools per hour), 10 schools per hour at wind farm PILE (N = 246, range = 0-106 schools per hour) and 7 schools per hour at wind farm CONTROL (N = 251, range = 0-37 schools per hour). The median number of schools inside and outside the wind farm was roughly equal for wind farms SEISMIC (2 schools per hour inside and out-

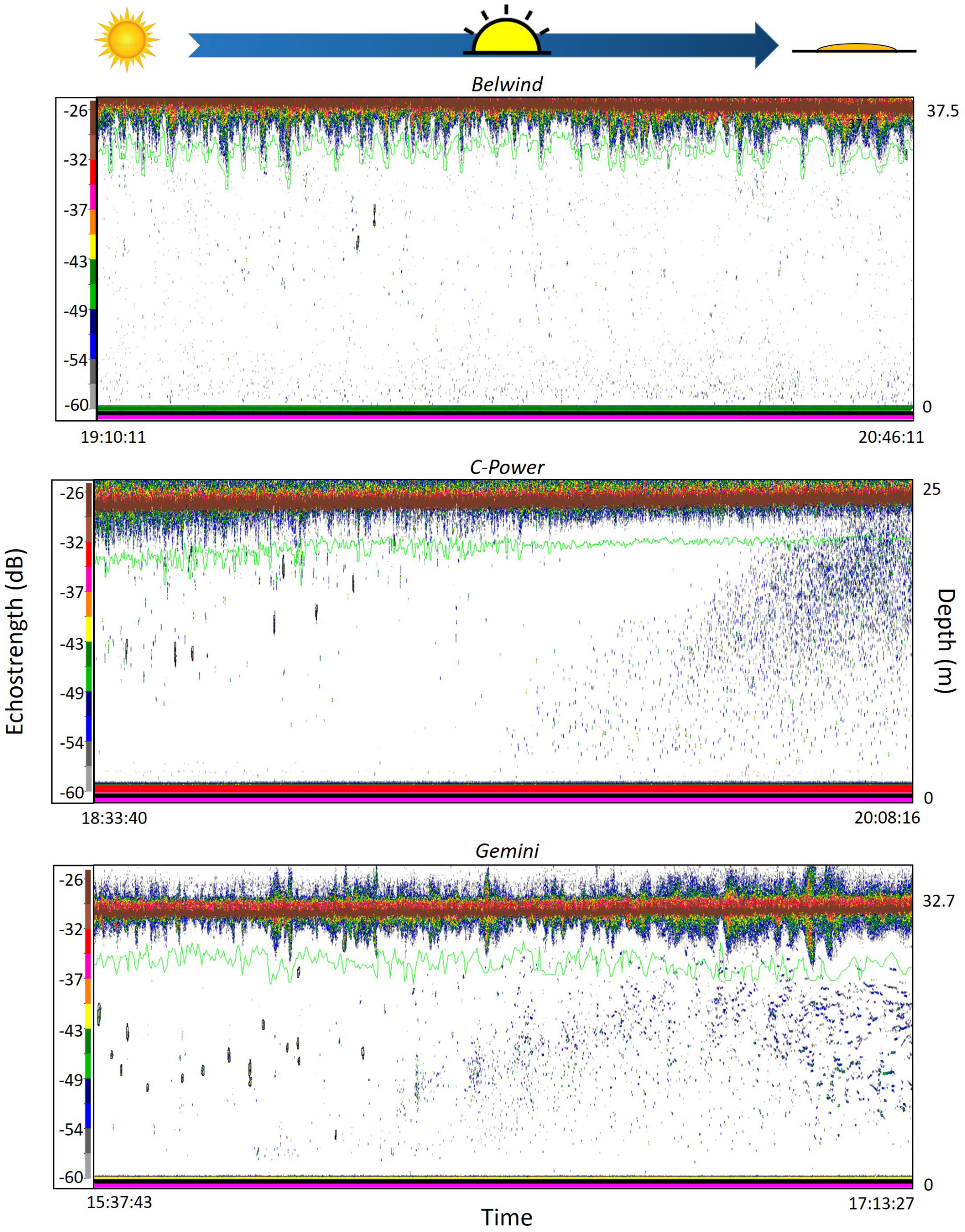

Figure 4: AZFP echosounder view for the period around dusk at the three locations. At wind farms PILE and CONTROL (middle and bottom panel), fish schools dispersed at dusk, with individual fish using the entire water column (PILE) or forming a layer in the middle of the water column (CONTROL). At wind farm SEISMIC, the schooling pattern did not change over the day.

Table 2: the biomass depth was influenced by several factors, as shown by general linear models of wind farm SEISMIC, wind farm PILE and wind farm CONTROL data. Significant factors were found by bootstrapping estimate values. If the 95% CI did not cross zero, factors were considered of significant influence (indicated with an asterisk). Note that since the models used a Gamma distribution, estimate values have to be converted before comparing to the data.

| **Wind farm** | **Variable** | **2.5% limit estimate** | **97.5% limit estimate** | **5% limit estimate** | **95% limit estimate** |
|---|---|---|---|---|---|
| SEISMIC | Intercept* | 0.086 | 0.21 | 0.095 | 0.20 |
| | *Treatment* | | | | |
| | Exposure* | 0.0065 | 0.035 | 0.0087 | 0.033 |
| | *Location* | | | | |
| | Inside* | 0.0099 | 0.021 | 0.011 | 0.020 |
| | Wave height* | -0.017 | -0.0081 | -0.016 | -0.0088 |
| | Temperature* | -0.0078 | -0.0010 | -0.0071 | -0.0015 |
| | Total biomass | $-2.87*10^{-5}$ | $9.5*10^{-6}$ | $-2.72*10^{-5}$ | $4.38*10^{-6}$ |
| | Treatment:Location | -0.037 | 0.0056 | -0.033 | 0.002 |
| PILE | Intercept* | 0.13 | 0.28 | 0.14 | 0.27 |
| | *Treatment* | | | | |
| | Exposure | -0.013 | 0.0066 | -0.012 | 0.0043 |
| | *Location* | | | | |
| | Inside* | 0.0046 | 0.013 | 0.0053 | 0.012 |
| | Wave height* | 0.012 | 0.021 | 0.013 | 0.020 |
| | Temperature* | -0.012 | -0.0039 | -0.011 | -0.0043 |
| | Tide* | -0.012 | -0.0054 | -0.012 | -0.0060 |
| CONTROL | Intercept* | 0.11 | 0.20 | 0.12 | 0.19 |
| | *Treatment* | | | | |
| | Exposure | -0.0073 | 0.019 | -0.0049 | 0.017 |
| | Temperature* | -0.011 | -0.0018 | -0.010 | -0.0025 |
| | Tide* | 0.00057 | 0.022 | 0.0022 | 0.020 |

side) and CONTROL (6 inside, 7 outside). For wind farm PILE, there were considerably more schools outside the wind farm (17 schools) than inside (7 schools).

Abiotic characteristics influenced almost all biotic variables that were measured. Temperature negatively influenced the biomass depth, with the mean biomass being closer to the bottom when temperatures were higher (Table 2). Wave height led to deeper swimming schools, as well as influencing biomass depth, the number of schools per hour and school cohesion, although patterns for these other variables were not always consistent between wind farms (Tables 2-5). The third abiotic characteristic, tide, influenced all variables (Tables 2-6). Tidal influences were consistent for the abundance of schooling fish, which was higher at low tide than at high tide for all wind farms and for school cohesion, which was lower at low tide for two out of three wind farms.

Table 3: model results of number of schools per hour. Note that covariate estimates were produced using a negative binomial (Count) and binomial (zero) Hurdle model, so the estimate values have to be converted before comparing to the data.

| **Wind farm** | **Model part** | **Variable** | **2.5% limit estimate** | **97.5% limit estimate** | **5% limit estimate** | **95% limit estimate** |
|---|---|---|---|---|---|---|
| SEISMIC | Count | Intercept* | 0.74 | 1.76 | 0.85 | 1.69 |
| | | *Period* | | | | |
| | | During* | -0.74 | -0.018 | -0.68 | -0.075 |
| | | After | -0.35 | 0.39 | -0.28 | 0.33 |
| | | *Location* | | | | |
| | | Outside+ | -0.60 | 0.0048 | -0.56 | -0.044 |
| | | Wave height | -0.64 | 0.24 | -0.57 | 0.17 |
| | | Tide | -0.19 | 0.35 | -0.15 | 0.31 |
| | Zero | Intercept* | 0.36 | 1.38 | 0.44 | 1.28 |
| | | *Period* | | | | |
| | | During | -0.72 | 0.27 | -0.63 | 0.20 |
| | | After | -0.086 | 0.11 | -0.78 | 0.033 |
| | | *Location* | | | | |
| | | Outside | -0.088 | 0.68 | -0.024 | 0.63 |
| | | Wave height* | 0.072 | 1.36 | 0.15 | 1.22 |
| | | Tide+ | -0.64 | 0.011 | -0.58 | -0.043 |
| PILE | Count | Intercept* | 2.80 | 3.90 | 2.89 | 3.81 |
| | | *Period* | | | | |
| | | During | -0.57 | 0.12 | -0.51 | 0.069 |
| | | After* | -4.68 | -2.55 | -4.35 | -2.69 |
| | | *Location* | | | | |
| | | Outside* | 0.16 | 0.72 | 0.20 | 0.68 |
| | | Wave height* | -0.95 | -0.16 | -0.88 | -0.22 |
| | | Tide* | 0.095 | 0.61 | 0.14 | 0.56 |
| | Zero | Intercept* | 2.24 | 20.34 | 2.52 | 10.75 |
| | | *Period* | | | | |
| | | During* | -17.99 | -0.058 | -4.93 | -0.34 |
| | | After* | -20.95 | -2.85 | -8.96 | -3.08 |
| | | *Location* | | | | |
| | | Outside* | 0.12 | 2.95 | 0.32 | 2.58 |
| | | Wave height | -2.55 | 1.30 | -2.16 | 0.97 |

Table 3 continued.

| **Wind farm** | **Model part** | **Variable** | **2.5% limit estimate** | **97.5% limit estimate** | **5% limit estimate** | **95% limit estimate** |
|---|---|---|---|---|---|---|
| CONTROL | Count | Intercept* | 1.36 | 1.90 | 1.40 | 1.86 |
| | | *Period* | | | | |
| | | During* | 0.32 | 0.93 | 0.37 | 0.88 |
| | | After* | 0.19 | 0.79 | 0.24 | 0.74 |
| | | *Location* | | | | |
| | | Outside | -0.086 | 0.34 | -0.048 | 0.31 |
| | | Tide | -0.39 | 0.11 | -0.35 | 0.066 |
| | Zero | Intercept* | 1.82 | 4.69 | 1.95 | 4.34 |
| | | *Period* | | | | |
| | | During$^{+}$ | -0.10 | 2.40 | 0.090 | 2.08 |
| | | After* | 0.22 | 4.34 | 0.42 | 3.73 |
| | | *Location* | | | | |
| | | Outside* | -3.21 | -0.38 | -2.89 | -0.54 |
| | | Tide | -4.71 | -0.99 | -4.20 | -1.20 |

Table 4: Covariate estimates influencing school depth at wind farm SEISMIC, wind farm PILE and wind farm CONTROL. Covariate estimates were converted back, so can be used without modification.

| **Wind farm** | **Variable** | **2.5% limit estimate** | **97.5% limit estimate** | **5% limit estimate** | **95% limit estimate** |
|---|---|---|---|---|---|
| SEISMIC | Intercept* | -148.87 | -101.46 | -145.01 | -105.49 |
| | Period | | | | |
| | During+ | -0.015 | 1.64 | 0.11 | 1.50 |
| | After* | 4.36 | 5.93 | 4.48 | 5.81 |
| | Location | | | | |
| | Outside* | -1.51 | -1.29 | -1.23 | -1.25 |
| | School Height* | 0.25 | 0.86 | 0.29 | 0.82 |
| | Wave height* | -4.68 | -2.54 | -4.50 | -2.72 |
| | Tide* | 3.26 | 4.52 | 3.37 | 4.42 |
| PILE | Intercept* | 17.69 | 25.63 | 18.44 | 25.07 |
| | Period | | | | |
| | During* | 6.90 | 7.83 | 6.97 | 7.76 |
| | After | -2.47 | 7.06 | -1.12 | 6.73 |
| | Location | | | | |
| | Outside* | 6.64 | 7.54 | 6.68 | 7.36 |
| | School Height | -1.17 | 2.62 | -0.89 | 2.49 |
| | Wave height* | -9.23 | -8.07 | -9.14 | -8.18 |
| | Tide+ | -3.72 | 0.65 | -3.56 | 0.85 |
| CONTROL | Intercept* | 5.25 | 6.92 | 6.03 | 6.76 |
| | Period | | | | |
| | During* | 1.48 | 1.86 | 1.51 | 1.82 |
| | After* | 1.15 | 1.45 | 1.17 | 1.41 |
| | Location | | | | |
| | Outside* | 1.12 | 1.06 | -1.05 | -1.05 |
| | School Height* | 1.16 | 1.21 | 1.16 | 1.21 |
| | Wave height | -1.01 | 1.072 | -1.0042 | 1.07 |
| | Interaction | | | | |
| | During:Outside* | -1.40 | -1.04 | -1.37 | -1.07 |
| | After:Outside | -1.40 | 1.09 | -1.38 | 1.06 |

Table 5: Covariate estimates influencing school cohesion at wind farm SEISMIC, wind farm PILE and wind farm CONTROL. Note that covariate estimates were calculated using a Gamma-distributed GLM and have to be converted before comparing to the data.

| Wind farm | Variable | 2.5% limit estimate | 97.5% limit estimate | 5% limit estimate | 95% limit estimate |
|---|---|---|---|---|---|
| SEISMIC | Intercept* | 0.033 | 0.042 | 0.034 | 0.041 |
| | Period | | | | |
| | During* | 0.00021 | 0.00059 | 0.00023 | 0.00057 |
| | After* | 0.00082 | 0.00012 | 0.00086 | 0.0012 |
| | Location | | | | |
| | Outside* | 0.00048 | 0.00052 | 0.00062 | 0.00056 |
| | School Height* | 0.00023 | 0.00036 | 0.00024 | 0.00035 |
| | Wave height$^{+}$ | -0.00048 | $8.2 *10^{-7}$ | -0.00044 | $-2.4 *10^{-5}$ |
| | Tide* | -0.00060 | -0.00039 | -0.00059 | -0.00040 |
| | Interaction | | | | |
| | During:Outside | -0.00087 | -0.00028 | -0.00082 | -0.00032 |
| | After:Outside | -0.0012 | -0.00066 | -0.0012 | -0.00070 |
| PILE | Intercept* | 0.0010 | 0.0060 | 0.0014 | 0.0056 |
| | Period | | | | |
| | During* | 0.00051 | 0.00052 | 0.00024 | 0.00049 |
| | After* | -0.0031 | -0.0013 | -0.0029 | -0.0014 |
| | Location | | | | |
| | Outside* | -0.00043 | -0.00060 | -0.00046 | -0.00051 |
| | School Height* | 0.00036 | 0.00051 | 0.00037 | 0.00050 |
| | Tide* | 0.00054 | 0.00075 | 0.00056 | 0.00073 |
| | Interaction | | | | |
| | During:Outside* | -0.00062 | -.000023 | -0.00059 | -0.00026 |
| | After:Outside* | 0.0016 | 0.0030 | 0.0020 | 0.0011 |
| CONTROL | Intercept* | 0.0026 | 0.019 | 0.0041 | 0.018 |
| | Period | | | | |
| | During$^{+}$ | -0.00055 | 4.31 | -0.0050 | $-2.86 *10^{-6}$ |
| | After | -0.00015 | 0.00043 | -0.00010 | 0.00039 |
| | Location | | | | |
| | Outside* | -0.00069 | -0.00049 | -0.00059 | -0.00056 |
| | School Height* | $5.43 *10^{-5}$ | 0.00020 | $6.54 *10^{-5}$ | 0.00019 |
| | Tide* | $3.35 *10^{-5}$ | 0.00054 | $7.42 *10^{-5}$ | 0.00050 |

Table 6: Covariate estimates influencing school biomass per hour at wind farm SEISMIC, wind farm PILE and wind farm CONTROL. Note that covariate estimates were calculated using a 10-log-distributed GLM and have to be converted before comparing to the data.

| **Wind farm** | **Variable** | **2.5% limit estimate** | **97.5% limit estimate** | **5% limit estimate** | **95% limit estimate** |
|---|---|---|---|---|---|
| SEISMIC | Intercept* | 5.15 | 14.78 | 5.91 | 13.99 |
| | Period | | | | |
| | During | -0.25 | 0.14 | -0.21 | 0.11 |
| | After | -0.50 | 0.18 | -0.17 | 0.14 |
| | Tide* | -0.39 | -0.13 | -0.37 | -0.15 |
| | Wave Height | -0.41 | 0.066 | -0.37 | 0.030 |
| PILE | Intercept* | 2.62 | 11.09 | 3.30 | 10.35 |
| | Period | | | | |
| | During | -0.19 | 0.32 | -0.15 | 0.28 |
| | After | -0.57 | 0.19 | -0.51 | 0.12 |
| | Wave Height | -0.15 | 0.36 | -0.11 | 0.32 |
| | Tide* | -0.46 | -0.10 | -0.42 | -0.13 |
| CONTROL | Intercept* | 0.91 | 20.74 | 2.55 | 19.10 |
| | Period | | | | |
| | During$^{+}$ | -0.56 | 0.034 | -0.51 | -0.0091 |
| | After | -0.51 | 0.12 | -0.45 | 0.067 |
| | Tide* | -0.62 | -0.016 | -0.57 | -0.067 |

## *Wind farm SEISMIC*

During the seismic exposure, the biomass depth outside the wind farm was significantly shallower than during the baseline (Fig. 5a & b; Table 2). Fish school presence also changed during the exposure by the seismic survey (Fig. 5c & d; Table 3): fewer fish schools were present than before or after the exposure, although the abundance of schooling fish did not change (Table 6). This indicates that fish were present in fewer, but larger schools during the seismic survey. The probability that a school was present did not change between periods, i.e. there was no change in the number of hours with schools present. This was matched by the non-significant result of the rotation test ($p>0.1$). The fish schools that were present during exposure tended to swim shallower (non-significant trend; Fig. 5e; Table 4) and schools inside the wind farm were more cohesive (Fig. 5f; Table 5). After the seismic exposure, schools inside the wind farm were also more cohesive than before the exposure and schools both inside and outside the wind farm swam higher in the water column.

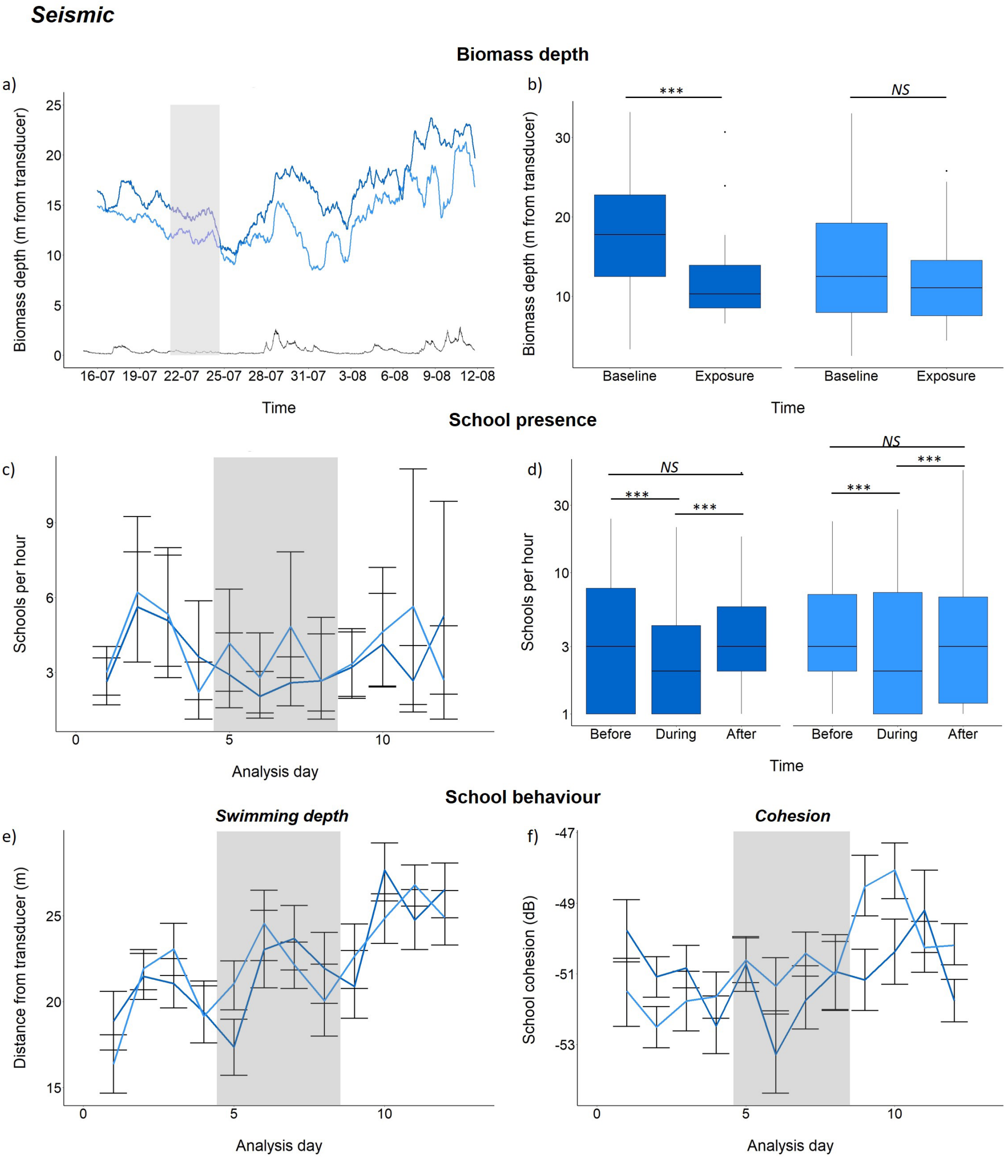

Figure 5: The seismic survey correlated with changes in a & b) biomass depth, c & d) number of fish schools per hour, e) school swimming depth and f) school cohesion. Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods. Error bars (c, e, f) depict bootstrapped 95% CI. Boxplots (b, d) show median (black line), first and third quartile (box) and 1.5 inter-quartile range (whiskers). Dots represent any data point outside of this range. Biomass depth is depicted as a) rolling mean (window length: 24 h), with wave height (black line) and b) taken every 3 hours during baseline and exposure.

### *Wind farm PILE*

The biomass depth was not significantly different from baseline, inside or outside, during the exposure to pile driving (Fig. 6a & b; Table 2). On each day of exposure, the probability of school detection in the actual hours of exposure also did not differ from the probability in the other hours of that day (rotation tests >0.1). However, the number of fish schools per hour as well as the probability that a school was present decreased in the AFTER period, without a change in abundance of schooling fish (Fig. 6c & d; Table 6). In the DURING period, schools were present higher in the water column, but recovered after the exposure (Fig. 6e; Table 4). Inside the wind farm, school cohesion was significantly higher in the DURING period compared to BEFORE, while it decreased again AFTER exposure and was lower than BEFORE the exposure period (Fig. 6f; Table 5). Outside wind farm PILE, however, there were no significant differences in cohesion between the periods.

### *Wind farm CONTROL*

At wind farm CONTROL, the biomass depth was not significantly different from baseline, inside or outside the wind farm (Fig. 7a & b; Table 2). However, the number of fish schools significantly increased in the DURING period (Fig. 7c & d; Table 3). No other significant factors were detected by the model for fish school numbers. The rotation test did not indicate a significant change in school presence pattern (p>0.1). The abundance of schooling fish tended to be lower in the DURING, but not the AFTER period (Table 6). Combined with the results on the number of fish schools per hour, this means that there were more, similar-sized schools in the DURING period. Also, DURING schools swam higher in the water column compared to BEFORE as well as AFTER, and tended to be less cohesive (Fig. 7e & f; Table 4 & 5). In the AFTER period, school cohesion was the same as in the BEFORE period.

## Discussion

Our results provide detailed insight into the presence, schooling behaviour, and swimming depth of pelagic fish in relation to seismic and pile driving exposure. Exposure to the seismic survey was related to a deeper biomass centre of gravity (outside wind farm), higher school cohesion (inside the wind farm) and lower school numbers (both inside and outside wind farm). Pile driving was related to shallower and more cohesive schools (inside the wind farm). In contrast, during the no-exposure period at the control site more schools were present, which swam higher in the water column and which were less cohesive.

Generally, we found very similar patterns within and outside the wind farm. This suggests that the environmental conditions of the wind farm itself did not change baseline behaviour of the pelagic fish community. Other abiotic conditions, in particular wave height and tidal differences, affected almost all of the parameters observed. Hence, these have to be considered when studying the responsiveness of fish schools to noisy human activities. However, the current data set shows that bottom-mounted AZFP-echosound-

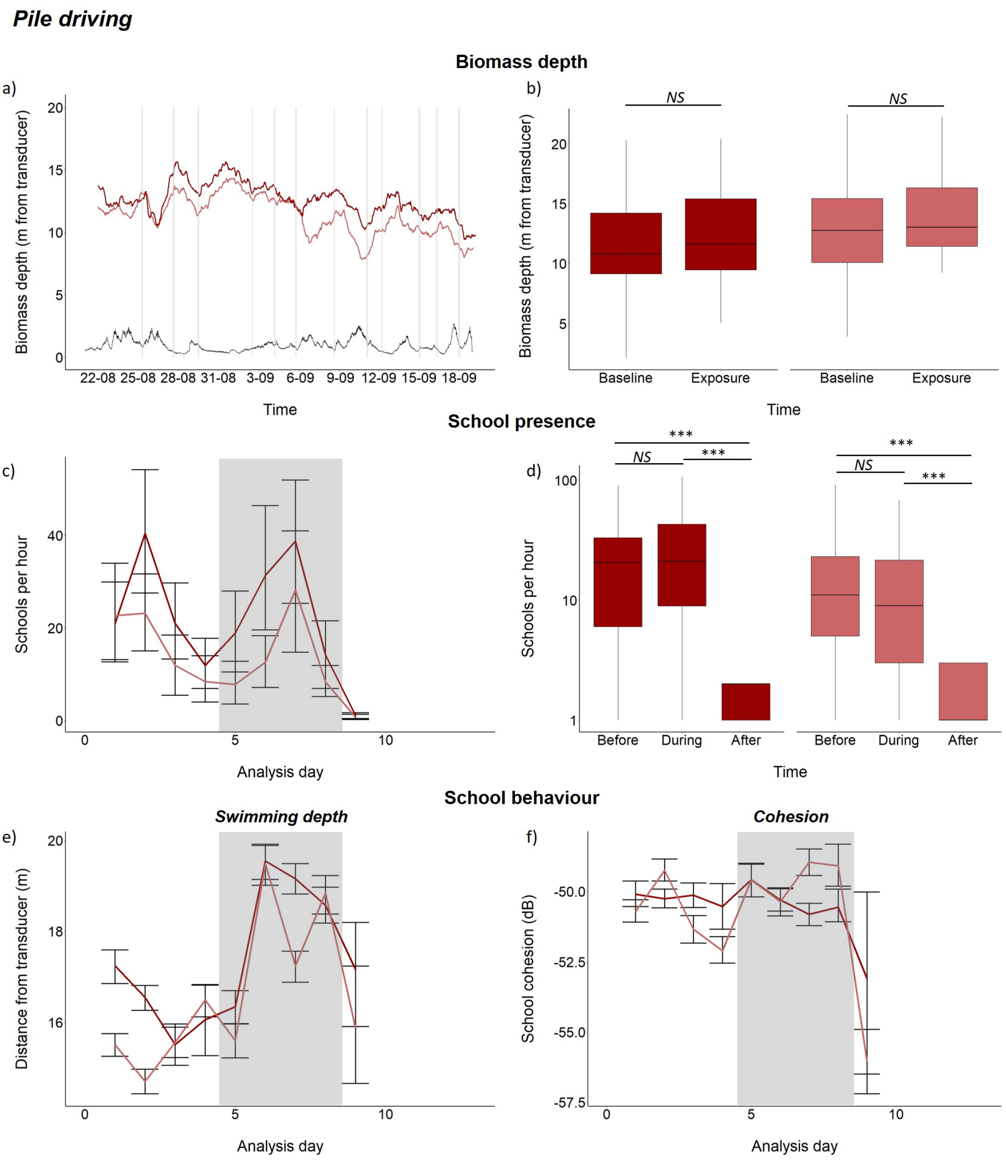

Figure 6: When fish were exposed to pile driving, there were no changes in a & b) biomass depth and c & d) number of fish schools per hour. The number of fish schools per hour did change after exposure. During exposure, school behaviour changed with schools e) swimming shallower and f) being more cohesive (only inside the wind farm). Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods (note the 12 brief exposure periods for the biomass depth in thin lines). Error bars (c, e, f) depict bootstrapped 95% CI. Boxplots (b, d) show median (black line), first and third quartile (box) and 1.5 inter-quartile range (whiskers). Dots represent any data point outside of this range. Biomass depth is depicted as a) rolling mean (window length: 24 h), with wave height (black line) and b) taken every 3 hours during baseline and exposure.

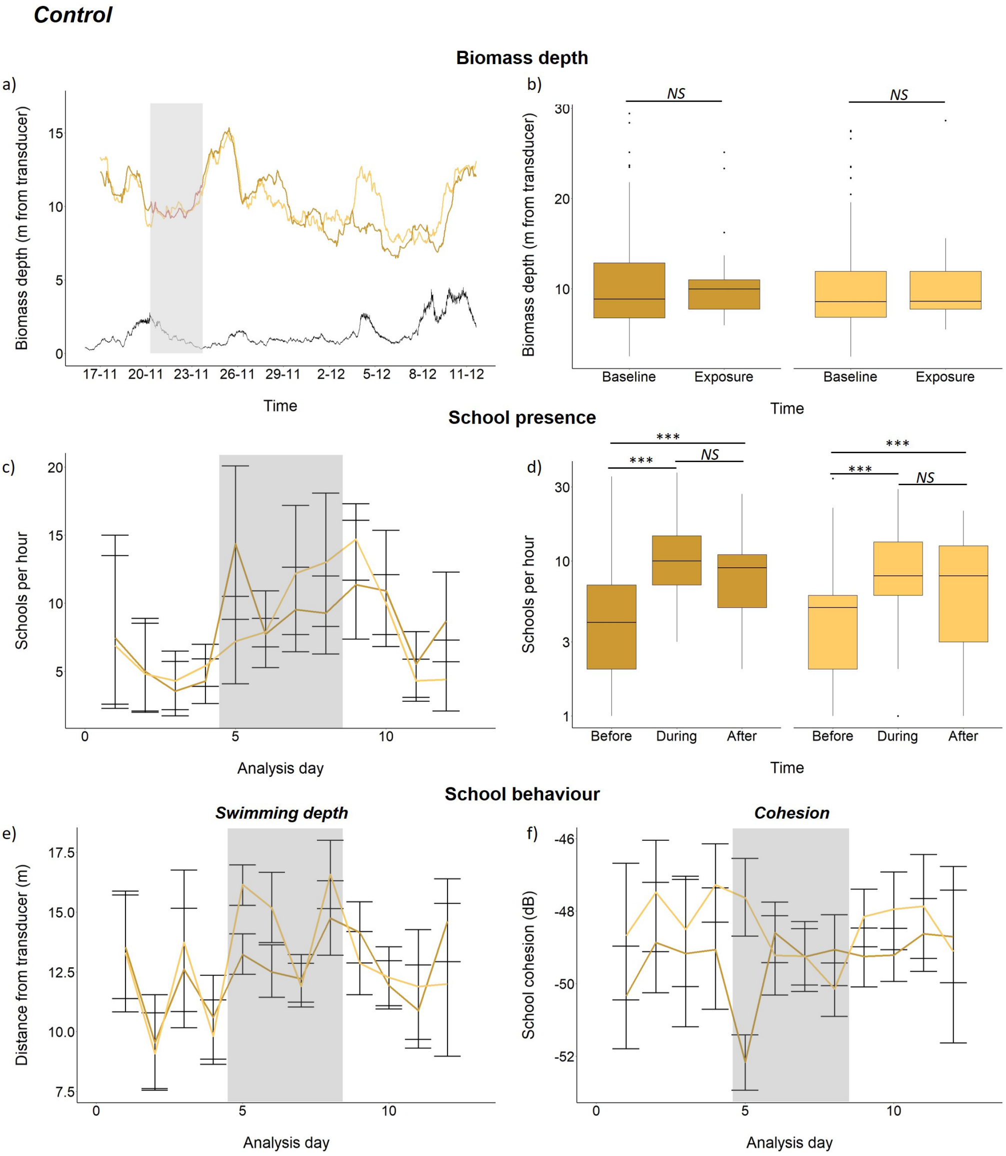

Figure 7: At wind farm CONTROL, there were no changes in a & b) biomass depth during the no-exposure period. The number of fish schools per hour (c & d) did change in the DURING period. Also, in the DURING period fish schools e) swam shallower inside the wind farm and f) were less cohesive. Dark coloured lines and boxes represent the AZFP outside the wind farm, light coloured lines and boxes represent the AZFP inside the wind farm. Shaded areas depict exposure periods (note the 12 brief exposure periods for the biomass depth in thin lines). Error bars (c, e, f) depict bootstrapped 95% CI. Boxplots (b, d) show median (black line), first and third quartile (box) and 1.5 inter-quartile range (whiskers). Dots represent any data point outside of this range.

ers are suitable to assess patterns of variation in sufficient detail to detect sound event related changes in presence, group cohesion, and swimming depth in the pelagic fish community, as shown for the data from wind farm SEISMIC and wind farm PILE. The current data set thus provides an unreplicated proof of concept. Finding proof for causal explanations for the correlations between exposure conditions and associated changes in fish schooling behaviour requires replication at multiple sites.

### *Changes in fish school behaviour related to sound exposure*

The number of fish schools per hour significantly decreased during exposure to the seismic survey. Earlier studies on reduction in catch rates and changes in fish abundance found varying results. Effects of seismic surveys on catch rates have been difficult to disentangle from the inherent variability in catch rates due to natural fluctuations (Parry and Gason 2006; Thomson et al. 2014; Bruce et al. 2018). Direct observations of reef fish abundance before and during a seismic survey nearby showed a marked decrease in the number of fish present, but mostly in the evenings (Paxton et al. 2017). The results of our study are in line with these earlier findings: the sound event-related decrease in fish schools is suggestive for fish leaving the area or altering their behaviour such that they become invisible for the echosounder. However, proper replication is required for drawing any conclusions about sound event related deterrence.

Sound events were related to changes in schooling behaviour with fish schools going up in the water column in response to both the seismic survey and the pile driving, although they also went up at the control site. At the same time, the mean depth of the total biomass went down in the water column during exposure to the seismic survey. Interestingly, our results on swimming depth of fish schools do not follow the general pattern found in literature of fish diving to deeper water upon acoustic disturbance (Slotte et al. 2004; Doksæter et al. 2012; Fewtrell and McCauley 2012; Hawkins et al. 2014b; Neo et al. 2014). However, there are also some studies that report fish swimming higher in the water column, either during or immediately after exposure (Chapman and Hawkins 1969; Sarà et al. 2007; Neo et al. 2015b). The discrepancy between the patterns found for fish schools and the total pelagic biomass is difficult to explain. It could be caused by behavioural differences in schooling fish compared to other species that make up the pelagic biomass, but this would have to be verified in future research.

School cohesion became higher during both sound exposures, while it decreased at the control site. Typically, fish schools initially decrease cohesion with a sudden exposure, followed by increased school cohesion (Doksæter et al. 2012; Fewtrell and McCauley 2012; Hawkins et al. 2014b; Neo et al. 2014; Neo et al. 2015b). Since the reports in the literature are typically observations over brief time periods (minutes to hours), while we report a response pattern analysed at a resolution of days, the increased school cohesion found matches with what would be expected for long-term responses of fish schools to sound. The consistency of this pattern between both exposure sites suggests that school cohesion is a variable that should be measured in any future investigations into effects of sound exposure that lasts for longer periods of time.

### *Effect of abiotic conditions*

Fish behaviour was affected by wave height and water temperature, as well as tide in some cases. We found that fluctuations in wave height affected swimming depth and school cohesion, with fish shifting down and (at wind farm SEISMIC) becoming more coherent schools in rough weather. Such weather dependent patterns have been reported before (e.g. Kaartvedt et al. 2017) and have been attributed to be a response to a decreased visibility (Tsuda et al. 2006) and a destratification of the water column (Secor et al. 2019). These patterns have further been correlated to increased wind speed (Lagardère et al. 1994) and a drop in barometric pressure (Heupel et al. 2003).

Another interesting pattern was distinct variation in schooling behaviour between day and night. Typical nocturnal behaviour with a release of clustering in schools to spread out individually across the water column was found for wind farm PILE and wind farm CONTROL, but not for wind farm SEISMIC. The dominant fish species may have been different for the different wind farms in the sampling periods and species may vary in their tendency to break up schools nocturnally. However, an alternative explanation is that wind farm SEISMIC was sampled first in the summer with long daylight periods, while wind farm PILE and wind farm CONTROL were sampled later in the autumn to winter, with already much shorter days and more distinct nocturnal parts of the day.

### *Using echo sounders to monitor long-term changes in fish behaviour*

This study has shown the potential of echosounders for studying long-term changes in the behaviour of pelagic fish. The advantage of using bottom-moored echosounders over a longer period of time is that the behaviour can be studied at various time scales. In this study, we focussed on long-term changes in the order of days, but shorter-term changes, such as the transition from schooling to individual swimming at dusk, can be studied as well. Depending on how long a school is in view of the echosounder, it might even be possible to study behaviour at the scale of seconds to minutes, as has been done previously with echosounders that were not bottom-moored but towed from a small boat (Hawkins et al. 2014b).

Fish behaviour, especially schooling behaviour, can vary considerably with daylight, tide, wave height and temperature. This was evident from all the behavioural variables measured. Inherent variability in the data may lead to false positive or false negative results, for example if a natural peak in the data coincides accidentally with the exposure event. Therefore, it is important to use considerable replication for exposure and control sites, as well as allowing for enough baseline data per site. At the same time, data from the two echosounders placed in each wind farm were strikingly similar. This suggests that data from one echosounder may be representative of an area that is larger than the wind farm in which it is placed.

Further understanding of fish behaviour may be achieved when echosounder deployment is combined with bio-sampling to verify the species composition on site. Another consideration is the use of multi-beam echosounders or, for large water depths, an autonomous vehicle with a mounted echosounder (Chu 2011; Benoit-Bird et al. 2017). Multi-beam echosounders make it possible to study horizontal movement, while an autonomous vehicle can get close to fish schools and follow them over a longer period of time, thereby getting detailed information of the behaviour of a particular school. Regardless, bottom-moored single-beam echosounders, as used here, are very well capable of studying vertical movement behaviour of fish (an often observed change in response to sound) and long-term changes in fish school dynamics.

### *Conclusions*

We have shown that bottom mounted AZFP-echosounders are a very suitable method to monitor fluctuations in time and space in pelagic fish communities. Fish exposed to the seismic survey and pile driving were less abundant and swam shallower in more coherent schools during the exposure days than before the exposure. However, we refrain from drawing strong conclusions about a causal relationship here and we stress that these data concern case studies and serve as a proof of concept. The sound event related changes in fish density and schools are unreplicated samples of patterns that fluctuate in time naturally. We stress the importance of the well-replicated use of bottom-mounted echosounders for future studies, to gain a better understanding of the pelagic fish community, potential effects of wind farm ecology, and the impact of anthropogenic noise.

## Acknowledgments

We would like to thank all the people involved in data collection. Furthermore, we would like to thank Echoview Inc. for providing us with a temporary license of Echoview 9, which was incremental for analysing the data. This project was funded by Rijkswaterstaat (ViSZiON project). The seismic survey exposure was undertaken as part of a larger project, PCAD4Cod, which was funded by the Joined Industry Programme.

# Appendix

Table S1: overview of the measuring locations for the environmental variables: tide height, water temperature and wave height. All measurements were taken from www.waternet.nl (Rijkswaterstaat), an online governmental repository for (a)biotic measurements of Dutch water bodies. Data from measuring locations were chosen based on the proximity of the location to the wind farm, as well as similar conditions (e.g. a measuring location that was also in open water was taken for temperature measurements). For some variables, choice of the measuring location was restricted to a subsample of locations that had available data of the desired period.

| Wind farm | Variable | Measuring location |
|---|---|---|
| Wind farm SEISMIC & Wind farm PILE | Tide | Euro platform |
| | Temperature | Euro platform |
| | Wave height | Schouwenbank Anchor South (wrakkenboei) |
| Wind farm CONTROL | Tide | Platform F16-A |
| | Temperature | Borkum Noord |
| | Wave height | Schiermonnikoog Noord |

Table S2: GPS coordinates of deployment locations of AZFP echosounder frames at the three wind farms and distance between the outside and inside frames at each wind farm.

| Echosounder | Latitude | Longitude | Distance outside-inside (m) |
|---|---|---|---|
| Wind farm SEISMIC Outside frame | 51.68821 | 2.84921 | 3031 |
| Wind farm SEISMIC Inside frame | 51.66522 | 2.82558 | |
| Wind farm PILE Outside frame | 51.58611 | 3.01083 | 2347 |
| Wind farm PILE Inside frame | 51.56972 | 2.98944 | |
| Wind farm CONTROL Outside frame | 54.0102 | 5.94827 | 2443 |
| Wind farm CONTROL Inside frame | 54.01033 | 5.91089 | |

Table S3: Exposure periods for analysing biomass data and fish school data.

| **Wind farm** | **Actual exposure** | | **Exposure period biomass data** | | **Exposure period fish school data** | |
|---|---|---|---|---|---|---|
| | *Start* | *End* | *Start* | *End* | *Start* | *End* |
| SEISMIC | 21-07 04:22 | 24-07 16:16 | Actual exposure | | 21-07 00:00 | 24-07 23:59 |
| PILE | 26-08 06:00 | 26-08 08:25 | Actual exposure | | 26-08 00:00 | 26-08 23:59 |
| | 28-08 13:40 | 28-08 16:40 | | | 28-08 00:00 | 28-08 23:59 |
| | 30-08 10:30 | 30-08 13:15 | | | 30-08 00:00 | 30-08 23:59 |
| | 03-09 12:10 | 03-09 13:50 | | | 03-09 00:00 | 03-09 23:59 |
| | 05-09 03:45 | 05-09 05:45 | | | | |
| | 06-09 18:10 | 06-09 20:15 | | | | |
| | 09-09 15:10 | 09-09 16:45 | | | | |
| | 12-09 01:50 | 12-09 04:25 | | | | |
| | 13-09 04:55 | 13-09 07:00 | | | | |
| | 15-09 23:30 | 16-09 02:55 | | | | |
| | 17-09 06:55 | 17-09 09:35 | | | | |
| | 18-09 22:40 | 19-09 02:00 | | | | |
| CONTROL | None | | 20-11 06:30 | 23-11 17:00 | 20-11 00:00 | 23-11 23:59 |

Table S4: Settings for automatic school detection with Echoview, as used to process data from the two echosounders for each of the three locations.

| **Setting** | **Length (m)** | **Corresponding number of pings** |
|---|---|---|
| Min. Total length | 1 | 8.3 |
| Min. Total height | 1 | n/a |
| Min. Candidate length | 0.5 | 4.2 |
| Min. Candidate height | 0.5 | n/a |
| Max. Vertical linking distance | 1.2 | n/a |
| Max. Horizontal linking distance | 0.8 | 10 |

Table S5: Distributions used for modelling the effect of sound on biomass depth, schools per hour, school size, school depth and school cohesion.

| **Response variable** | **Wind farm** | **Model distribution** |
|---|---|---|
| Biomass depth | All | Gamma |
| Schools per hour | All | Zero-inflated negative binomial |
| School size | All | $\text{Log}_{10}$ transformation |
| School depth | SEISMIC | normal |
| | PILE | Square transformation |
| | CONTROL | $\text{Log}_{10}$ transformation |
| School cohesion | All | Gamma |

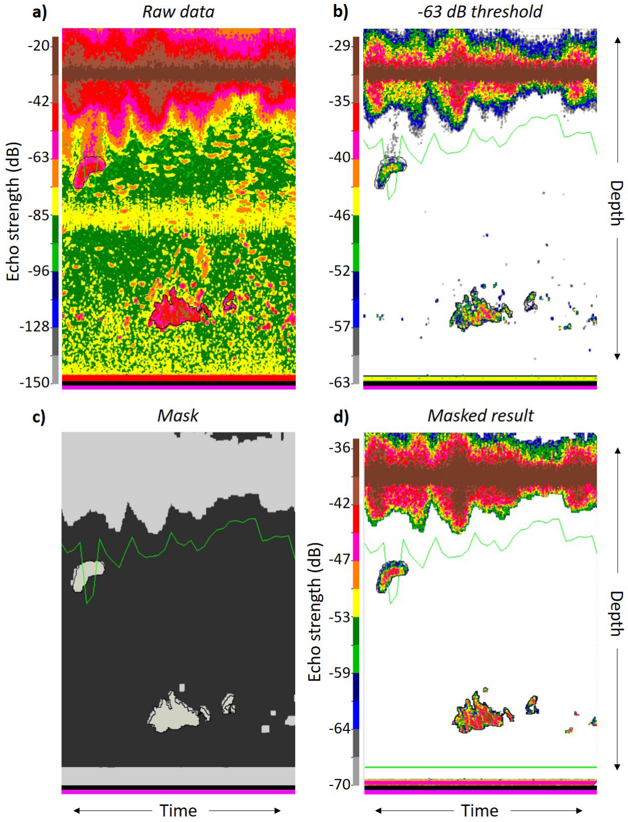

Figure S1: The raw echosounder data (a) was pre-processed to reduce noise and enhance the detectability of schools. Data of all echosounder frequencies were filtered with a -63-dB threshold (b) and combined to create a maximum strength echogram. Next, the combined data was eroded and dilated so only clusters of pixels were retained, of which a mask was created (c). The mask was then put over the raw data of 125 kHz, to create the filtered result that could be used for data analysis (d). Noise from the surface and the bottom was excluded from automatic school detection (green lines). Distinct schools that fell (partly) outside of the green lines were manually added to the detected schools.

# Effects of short-term and long-term noise exposure on anti-predator behaviour: A field experiment with sand gobies

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## Abstract

The oceans and seas are getting noisier due to human activities. In today's marine habitats, anthropogenic noise is widespread in space and time. This affects aquatic animal communities. As a result, most free-ranging animals are no longer naïve to noise pollution and may have habituated or become sensitized to particular forms of anthropogenic noise. The type and magnitude of responses at individual and species level affect species interactions and determine community level impact. However, how long-term exposure affects predation has never been tested. We investigated the interaction between short-and long-term sound exposure on the anti-predator response of free-ranging sand gobies (Pomatoschistus minutus), thus investigating behavioural alterations with the potential for direct fitness effect on both the prey and its predator. We explored long-term effects of noisy conditions by testing the response to a simulated predator strike in a range of sites that were more or less heavily exposed to boat sounds and experimentally tested the effects of short-term playback of boat noise. Individuals exposed to short-term playback of boat noise were absent from the experimental area for shorter periods after the predator strike than gobies exposed to ambient noise playback. Gobies in long-term noisy habitats also stopped avoiding the area after the predator strike under ambient noise conditions. These changes point to a decreased magnitude in anti-predator response, as a function of interacting short- and longer-term levels of disturbance. Thus, prey species alter their anti-predator behaviour in ways that can lead to potentially higher mortality for individual prey. This could ultimately lead to effects at population-level for both predator and prey, with potential implications at the level of the food web.

## Introduction

Noise pollution is omnipresent in today's terrestrial and marine habitats and may affect individual species, as well as changing species interactions and animal communities (Francis et al. 2009; Barber et al. 2010; Slabbekoorn et al. 2010; Kunc et al. 2016; Shannon et al. 2016; Slabbekoorn et al. 2019). Aquatic animals that are naïve to particular noisy human activities may be negatively affected through deterrence, disturbance, distraction, or through masking of biologically relevant sounds. Furthermore, repeated or continuous exposure to noise may cause long-term changes in animal behaviour, due to processes such as habituation or sensitisation (Bejder et al. 2009; Harding et al. 2018). Therefore, studies on noise impact should include the potential effects of habituation and sensitization to long-term sound exposure of non-naïve individuals. Currently, these studies are lacking, with experimental as well as observational studies on species interactions only focussing on effects of short-term or long-term exposure contexts separately (Voellmy et al. 2014b; Simpson et al. 2015; Nedelec et al. 2016; Neo et al. 2016; Radford et al. 2016; Simpson et al. 2016; Harding et al. 2019).

Repeated exposure to noise can either increase or diminish the behavioural response of the population. Behavioural responses may get stronger through sensitisation to the disturbance. This is often caused by noise that induces a startle reflex in animals (Götz and Janik 2011). Alternatively, the effect of noise on animals that are exposed repeatedly can diminish over time. The diminishing effect of repeated noise exposure can have two causes. First, animals living in disturbed areas may have become habituated to noise. Coral reef fish, for example, increased their hiding time when first exposed to boat noise, but no longer so following two weeks of exposure (Nedelec et al. 2016). Second, non-coping individuals may have left the disturbed area, leaving only individuals that are intrinsically more able to cope with the disturbance (Bejder et al. 2006a; Bejder et al. 2006b). A combination of increasing tolerance levels in coping individuals and mortality or displacement of non-coping individuals could thus lead to decreased effects of short-term noise exposure for individuals remaining in disturbed areas.

A specific effect of noise concerns the change in anti-predator behaviour of prey. Changes in anti-predator behaviour have a high potential to affect vital rates of the prey species, as a decrease in effectiveness of anti-predator response could mean death of the organism (e.g. Simpson et al., 2016). Effects of short-term noise exposure on anti-predator behaviour have been found across taxa, including fish (Voellmy et al. 2014b; Simpson et al. 2015; Simpson et al. 2016; McCormick et al. 2018), invertebrates (Chan et al. 2010; Hubert et al. 2018) and mammals (Kern and Radford 2016; Morris-Drake et al. 2016). Most studies showed changes in anti-predator response latency, using simulated predators. One study also included live predation events, showing that prey were indeed more easily caught by a predator under noisy conditions (Simpson et al. 2016). However, the direction of change differs between species: some prey show increased response latency under noisy conditions, while others show a decreased response latency.

Two mechanisms have been proposed to explain the change in anti-predator behaviour of prey under noise exposure: distraction by non-relevant stimuli (the distracted prey hypothesis; Chan and Blumstein, 2011; Rosa and Koper, 2018) and increased vigilance interpreted as being due to perceived threat by the noise (the risk-disturbance hypothesis; Frid and Dill, 2002). Both distraction and vigilance have been reported in experimental studies of predation risk under noise exposure, although for some studies the experimental set-up leaves room for other interpretations (distraction: Chan et al. 2010; Simpson et al. 2015; Morris-Drake et al. 2016; Simpson et al. 2016; McCormick et al. 2018, vigilance: Karp and Root 2009; Voellmy et al. 2014; Kern and Radford 2016; Shannon et al. 2016). Still, these reported changes were all for animals living in similarly quiet conditions, while the type and magnitude of the effect is likely to be different for individuals in disturbed vs non-disturbed areas (Bejder et al. 2009). To the best of our knowledge, combined effects of short- and long-term sound exposure on anti-predator behaviour have never been tested.

To study the effects of short-term and long-term noise on anti-predator behaviour, sand gobies (Pomatoschistus minutus) are a suitable study system. Sand gobies are benthic sit-and-wait predators, making them easily observable in the field. They are under a high predation pressure by fish and marine mammals (Magnhagen and Forsgren 1991; Leopold 2015), so changes in their anti-predator behaviour may have large consequences. Goby anti-predator behaviour has two stages. When a predator passes, most gobies freeze and rely on their ability to blend in with the sediment to escape detection. If, however, a predator comes too close, gobies will show a typical C-start flight response to escape a direct attack (Magnhagen and Forsgren 1991) or bury themselves in the sediment to avoid predation (Ehrenberg and Ejdung 2008). Gobies are also sensitive to sound, which may affect their behaviour (Codarin et al. 2009; Picciulin et al. 2010; de Jong et al. 2018; Blom et al. 2019). Therefore, it is likely that their anti-predator behaviour is also affected by noise.

Here, we investigated the effects of short-term boat noise on the anti-predator behaviour of free-ranging sand gobies (Pomatoschistus minutus) inhabiting areas across a range of low to high long-term noise disturbance levels. We investigated whether goby behaviour is affected by a simulated predator strike. If so, how does playback of boat noise affect a goby's anti-predator response (short-term effect)? And is the level of disturbance of the habitat and thereby level of the experience with noisy conditions related to a goby's anti-predator behavioural response (long-term effect)? Our results provide insight into how short-term and long-term noise exposure can interact to affect anti-predator behaviour.

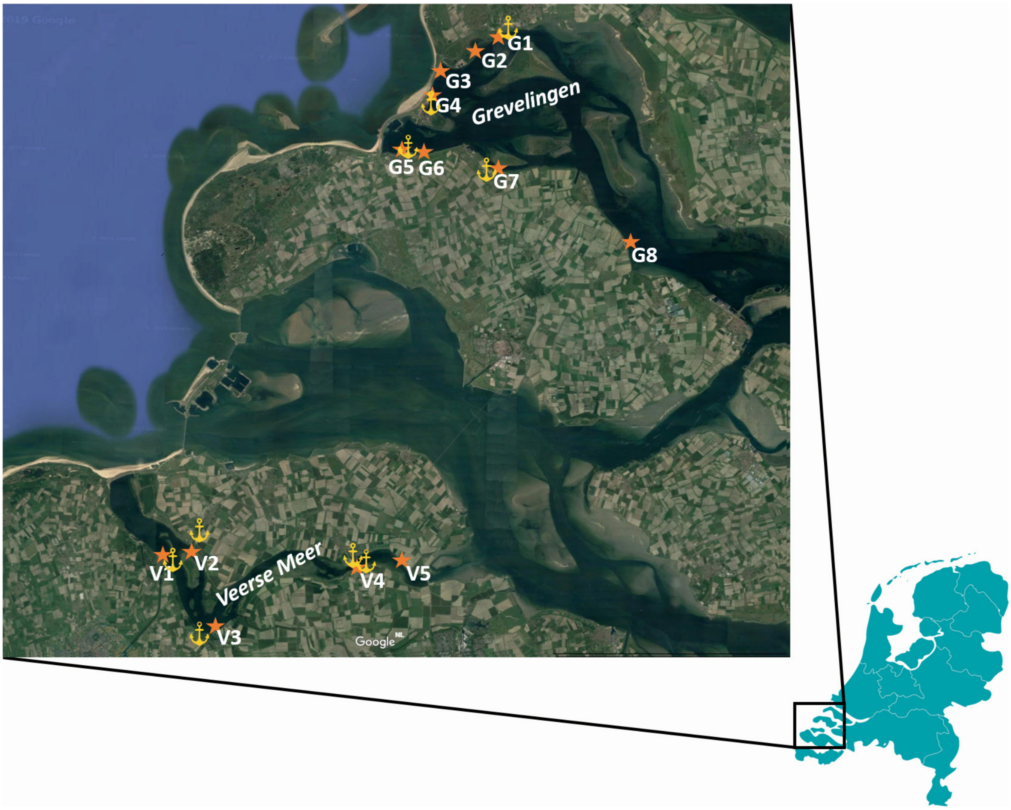

Figure 1. Experiment locations in Grevelingen (8) and Veerse Meer (5) (partially closed river ends). Locations were selected to include habitats with a gradient in the number of boats passing by per day and the mean ambient sound levels. Harbours near the experimental locations are indicated with a yellow anchor.

## MATERIALS AND METHODS

### Study site

All experiments were conducted April-June 2018 in two salt water lakes, Grevelingen and Veerse Meer, in Zeeland, the Netherlands (Fig. 1). In total, 13 locations were sampled, 8 in the Grevelingen and 5 in the Veerse Meer. Both lakes are in part connected to the North Sea, either directly or via an estuary, and maintain brackish (Veerse Meer) to salt (Grevelingen) conditions. Commercial shipping is rare, but recreational shipping is abundant, especially in summer. Most shipping activity centres around harbours, which leads to a patchy soundscape of quiet and noisy areas. Sand gobies are omni-present in the area.

### Soundscape

At all 13 locations, the soundscape was measured during a 22h daily cycle in the period of 30 April – 11 May 2019. Recordings were made using autonomous recorders (Sound

Traps, Ocean Instruments) at a sample rate of 192 kHz, and temperature and acceleration every 10 s. The recorders were stabilized in the water column using weights and a sub-surface float. Water depth at each location was 1.20-2 m. After retrieval, sound recordings were analysed for root-mean-square sound pressure level (SPLrms) during daylight and at night using PAMGuide (Merchant et al. 2015).

### *Experimental design*

Experiments consisted of exposing of free-ranging gobies to a simulated predatory attack (sensu Lorenz and Tinbergen, 1939), in combination with sound playback, according to a nested design. At each location, two playbacks were conducted, one with ambient and one with boat noise. The predator strike consisted of a model predatory fish (constructed from PVC tube, chicken wire and duct tape to have a streamlined front), mounted on an aluminium frame, that – when released - approached the location of the goby. The aluminium frame also supported two cameras to record the behaviour of the gobies and was moved between test locations (Fig. 2). Only gobies guarding nests (i.e. stationary location) were targeted to increase the probability that a goby would be in the video frame at the experimental release of the predator. The frame was placed such that the nest was in the centre of the frame. The playback speaker (Lubell UW30) was placed next to the frame at 1 m distance.

After placement and a 10-minute acclimatization period, the playback was started remotely, playing either boat engine sound (treatment) or ambient sound (control). After 5 minutes of playback, the predator was released remotely by releasing a 5 m rope. To ensure equal environmental conditions between treatments at the same location, one goby was exposed to ambient sound and another to boat sound at the same time. The

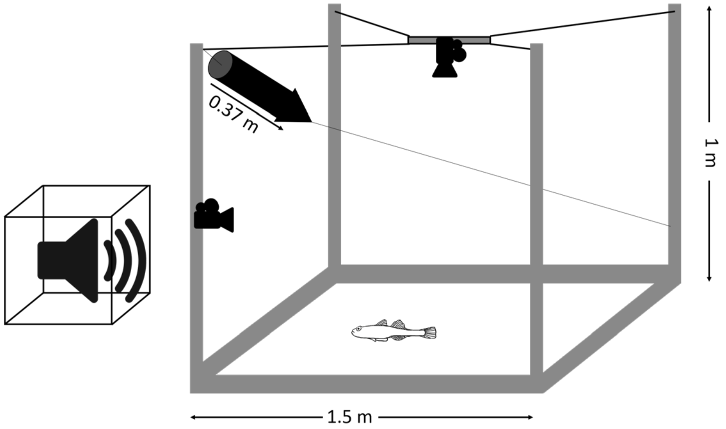

Figure 2. Schematic overview of the experimental setup. The construction was placed over a goby nest. After 10-minute acclimatisation, the speaker played either boat noise or ambient sounds for 10 minutes. After 5 minutes of playback, the simulated predator was released by releasing a rope from a distance of 5 m to slide across a fishing line in a simulated predator strike. Cameras recorded the goby's response.

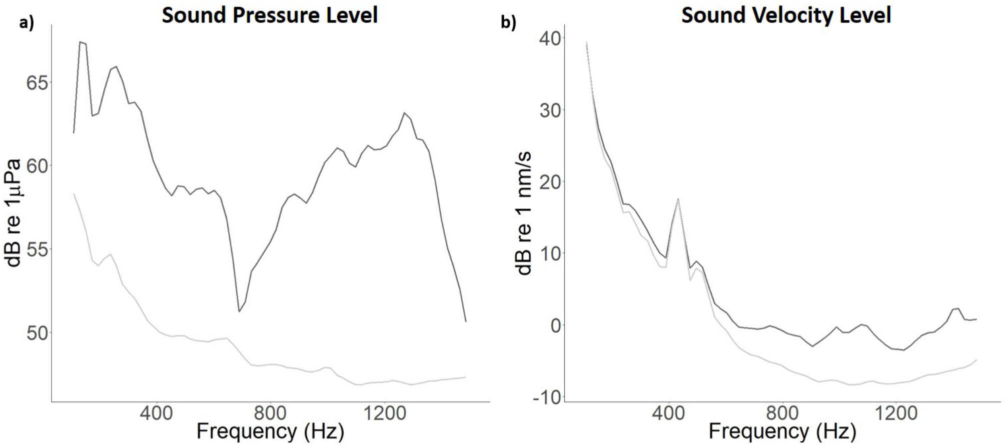

Figure 3. Average power spectral density (PSD) levels of all ambient (light grey) and boat (dark grey) playback sounds played back at 2 m water depth. PSD levels were measured for a) sound pressure and b) sound velocity.

two experimental frames were always placed at a distance of 15 m or more apart, because the playback sound did not propagate beyond 10 m (as verified with sound pressure recordings). For each location, we determined the SPLrms from the soundscape recordings.

### *Playback sounds*

Two types of sounds were broadcasted, boat engine sounds and ambient sounds (Fig. 3). All playback sounds were recorded with an AS1 hydrophone (Aquarian Scientific) with external amplifier, coupled to a handheld recorder (Tascam DR-100). Ambient sounds were recordings of the soundscape at the playback location. Sounds were high-pass filtered at 10 Hz to remove static noise from the hydrophone and were cleaned to remove sounds from passing ships and unusually loud sounds, e.g. from water movement. Speaker characteristics led to a high-pass cut-off frequency of 100 Hz in the field. All ambient sound playback stimuli were equalized at -50 dB. Boat sounds were recorded from passing boats at 5-20 m, at several locations in both lakes. Recordings with a section with a clear approach and exit, i.e. with a clear ramp-up and ramp-down of the sound, were selected and copied 5-10 times to produce a 15-minute playback stimulus (using Audacity 2.1.1). All boat sounds were equalized at -30 dB, because the average recorded level of boat sound was 20 dB higher than ambient. To avoid startle responses at the onset of the playback, a stimulus started with a 10 s fade in. To avoid pseudo-replication, a different version of the stimulus was used for each location.

We determined both sound pressure levels (SPL) and sound velocity levels (SVL) of played back boat and ambient sounds. SPL was measured using an AS1 hydrophone (Aquarian Scientific) with external amplifier, coupled to a handheld recorder (Tascam DR-100). SVL was measured with an M20 accelerometer (Geospectrum Technologies, Darmouth, Canada) coupled to an oscilloscope (Picoscope 3000, Pico Technology), that was connected to a laptop running a custom-made measuring programme (Campbell 2019). Playback sounds were recorded at 1 and 2 m depth, the range of water depths at which experiments were conducted, at ~1m from the speaker in the centre of the experimental arena. On average, played back boat sounds had a SPLrms of 98-101 dB re 1 µPa and a SVLrms of 83-84 dB re 1 nm, at 1-2m depth, respectively, comparable to boats passing by at 50-200 m (calculated using cylindrical spreading based on received levels of recorded boats). Ambient playback sounds did not reach above background noise levels, so the ambient noise playback was seen as a silent control.

### *Data analyses*

The goby type of anti-predator response could range from 1) freezing, a cryptic response when the predator is passing by, to 2) an active escape response, either a) a startle without displacement or b) leaving the site of attack. For each experiment we recorded the time a goby was frozen, whether or not it startled and the time it was away from the visible field of the camera (absent, i.e. left the immediate area of the nest) for 1 minute before and 1 minute after the predator strike. We conducted 23 experiments with ambient noise playback and 17 experiments with boat noise playback. All analyses were done blind to the experimental conditions. Behavioural analyses were blind to the start of the predator strike (not visible in the recording). The start of the predator strike was determined retrospectively by listening to the timing of the faint sound of the rope being released.

Video recordings of the experiments were analysed frame by frame, without the audio recording, using Kinovea (version 0.8.15). The time not frozen could include any movement from a subtle waving of the pectoral fin (to increase oxygen flow in the nest) to a distinct startle response (escape behaviour). Proportion of time frozen was calculated from dividing the time frozen by time in sight of the camera. If the goby left the field of view, it was recorded as having left for the rest of the sampling period, unless a goby coming back into view could be identified as the focal animal (i.e. by returning to its nest). We also measured response latency, another factor that is often found to change due to exposure to noise (Chan et al. 2010; Simpson et al. 2015; Simpson et al. 2016; McCormick et al. 2018). This was calculated as the time until the goby showed a change in behaviour, for example from swimming to frozen, after release of the predator.

### *Statistical analysis*

To investigate the effect of the sound playback on 1) proportion of time the individuals were frozen, 2) whether the gobies startled to the predator strike, 3) the proportion of time the gobies were absent and 4) response latency, we performed Generalized Linear Models (GLMs) with a beta distribution (link function = logit; Douma and Weedon 2019) and a binomial distribution for the startle response. For response latency, this required a zero-one-inflated beta distributed Monte Carlo Markov Chain (MCMC). Additionally, we performed a zero-inflated beta MCMC on the time gobies were absent before predator release.

Explanatory variables in the full models were treatment type (ambient or boat noise), SPLrms and the interaction between these two. For the response variable "proportion of time absent", we also included the time absent before predator release. For the response variable "response latency", an extra included explanatory variable was whether the goby startled. Due to some unsuccessful experiments, it was not possible to match ambient and boat sound treatments from the same day (i.e. with equal environmental conditions) for every location. Consequently, when comparing treatments, all ambient treatments were matched with all boat treatments within the same location. Model assumptions were checked by visual inspection of the predicted vs. the fitted residuals.

We calculated the change in proportion of time individuals were frozen and in the proportion of time they were absent between the period before predator release and the period after predator release. For time frozen, this was calculated as:

$$change = \frac{\frac{TimeFrozenAfter}{TimePresent} - \frac{TimeFrozenBefore}{TimePresent} + 1}{2} \quad (1)$$

For time the gobies were absent, the change was calculated as:

$$change = \frac{\frac{TimeAbsentAfter}{60} - \frac{TimeAbsentBefore}{60} + 1}{2} \quad (2)$$

Where *change* = 0 is the largest decrease in time frozen/time absent from view upon predator release, *change* = 0.5 means no change in time spent frozen/time absent, and *change* = 1 is the largest increase in time spent frozen/time absent post predator release.

The influence of the explanatory variables on the zero-one-inflated MCMC was tested using a likelihood ratio test from the lmtest package (Bolker et al 2015, lmtest ref). Final GLMs were selected using the dredge function from the MuMIn package which selects the best model based on corrected Akaike Information Criterion (AICc) score and circumvents order effects in model selection (MuMIn ref). After dredging, variable estimates were calculated by bootstrapping: the data set was resampled from the original data and estimates were calculated 10 000 times. If estimates of the explanatory variables did not cross zero in the 95% confidence interval (CI), explanatory variables were considered to be of significant influence on the response variable. All final models had normally distributed residuals.

## Results

### Habitat and experimental sound levels

Ambient SPLrms ranged between 68-96 dB re 1 μPa for the 13 locations (Fig. 4). In many locations, the SPLrms was enhanced by transient sounds, while the most often recorded ambient sound levels over the day were similar for all locations (Fig. 4). Both lakes had a range of quiet (e.g. 4a, c) to noisy (e.g. 4b, d) locations. Boat noise mostly increased sound levels above 200 Hz due to the shallow water depths in all areas (the cut-off frequency in 2 m water is ~765 Hz). Wave noise from boats and wind increased sound levels up to 300 Hz, but mostly in frequencies below 200 Hz.

### Time frozen

Gobies did not change the time they were frozen when the predator was released under the ambient noise treatment (Table 1). There was also no significant impact of either playback type or long-term SPL on the time gobies were frozen. However, gobies tended to increase their time frozen after predator release more under ambient than under boat

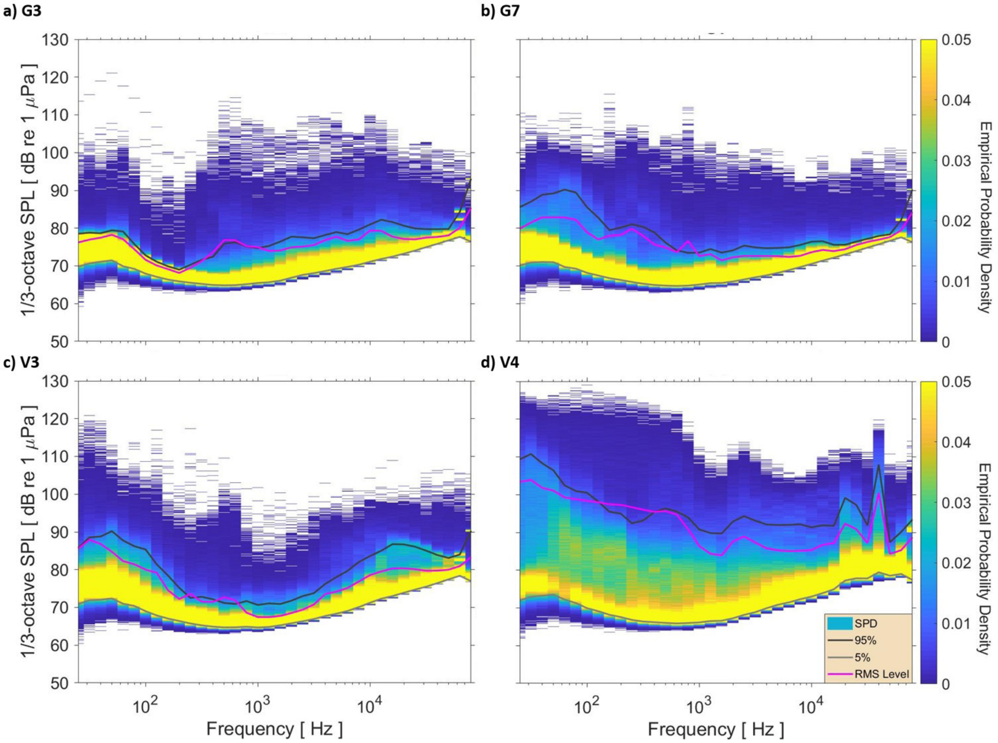

Figure 4. Probability densities of 1/3 octave SPLs of ambient sound at daytime at four of the thirteen experimental sites. Colours indicate the probability of a particular sound level occurring with warmer colours indicating a higher probability. Lines indicate 5 and 95% of all sound levels sampled (grey and black lines), and $SPL_{rms}$ levels (lilac line).

Table 1: Model results of the effect of playback type (ambient or boat noise) on the proportion of time a goby was frozen, whether a goby startled, the proportion of time a goby was in sight of the camera, and the response latency to the release of the predator. All models included one or more precision variables that described the variance of the posterior distribution of the model. The models for "Time absent before predator release" and "Response latency" separately modelled the probability of observing zero or zero and one. Significant explanatory variables are indicated with an asterisk (*) and non-significant trends are indicated with (+). Note that estimate values of the models for time frozen, time in sight and response latency were logit transformed because of the beta distribution and that estimate values of the model for startle were arcsine transformed because of the binomial distribution. Estimates should therefore be converted before comparing to the data.

| **Response variable** | **Explanatory variables** | **2.5% limit estimate** | **97.5% limit estimate** | **5% limit estimate** | **95% limit estimate** |
|---|---|---|---|---|---|
| Change in time frozen | Intercept | -5.85 | 0.75 | -5.40 | 0.35 |
| | Playback type (boat noise)$^+$ | -0.28 | 6.85 | 0.17 | 6.38 |
| | SPL | -0.0075 | 0.067 | -0.003 | 0.06 |
| | Playback type:SPL$^+$ | -0.079 | 0.0019 | -0.073 | -0.003 |
| | *Precision (φ)* | | | | |
| | Intercept* | 1.91 | 3.69 | 1.96 | 3.48 |
| | Playback type (boat noise)$^+$ | -0.20 | 2.59 | 0.049 | 2.36 |
| Startle | Intercept$^+$ | -1.95 | $7.16*10^{-17}$ | -1.73 | -0.11 |
| | Playback type (boat noise) | -0.42 | 2.48 | -0.16 | 2.20 |
| Change in time absent | Intercept* | 0.65 | 1.00 | 0.84 | 1.00 |
| | Playback type (boat noise)* | $4.45*10^{-6}$ | 0.24 | $1.03*10^{-5}$ | 0.10 |
| | SPL* | 0.47 | 0.50 | 0.48 | 0.50 |
| | Playback type:SPL* | 0.50 | 0.53 | 0.51 | 0.53 |
| | *Precision (φ)* | | | | |
| | Intercept* | 0.83 | 1.87 | 0.88 | 1.73 |
| | Playback type (boat noise)$^+$ | -0.15 | 3.03 | 0.02 | 2.70 |
| Time absent before pred. | Intercept | -12.28 | 11.35 | | |
| | Playback type (boat noise) | -4.55 | 23.27 | | |
| | SPL | -0.14 | 0.14 | | |
| | Playback type:SPL | -0.27 | 0.06 | | |
| | Probability of 0 (zi) | | | | |
| | Intercept* | 0.16 | 2.06 | | |
| | Playback type (boat noise)* | -2.92 | -0.12 | | |
| | *Precision (φ)* | | | | |
| | Intercept* | 0.84 | 2.87 | | |

Table 1 continued.

| Response variable | Explanatory variables | 2.5% limit estimate | 97.5% limit estimate | 5% limit estimate | 95% limit estimate |
|---|---|---|---|---|---|
| Response latency | Intercept | -5.73 | 5.23 | | |
| | Playback type (boat noise) | -7.55 | 4.43 | | |
| | SPL | -0.07 | 0.05 | | |
| | Startle (yes)* | -4.05 | -1.58 | | |
| | Playback type:SPL | -0.06 | 0.08 | | |
| | Playback type:Startle | -0.15 | 2.76 | | |
| | *Probability of 0 or 1 (zoi)* | | | | |
| | Intercept* | -2.03 | -0.12 | | |
| | Playback type (boat noise) | -2.20 | 0.99 | | |
| | *Precision (φ)* | | | | |
| | Intercept | -0.81 | 0.77 | | |
| | Playback type (boat noise)* | 0.08 | 2.30 | | |
| | Startle (yes)* | 2.25 | 4.46 | | |

noise playback (non-significant trend, Fig. 5a, Table 1). Furthermore, a higher ambient long-term SPL in the habitat tended to lead to a decreasing time spent frozen after predator release (non-significant trend, Fig. 5b).

### *Startle response*

Gobies showed no significant difference in probability of startling to the predator between ambient and boat noise playback (Table 1). Out of 23 and 17 gobies exposed to ambient and boat noise, 9 and 7 startled to the simulated predator, respectively. Long-term noise levels of the habitat did not influence this pattern either (SPLrms was excluded from the final model in model selection).

### *Time absent*

Gobies did, however, respond to the simulated predator by leaving their nest site. Under ambient noise playback, gobies spent significantly more time absent from the site after predator release, compared to before release (Fig. 5c; Table 1). This avoidance response was affected by long-term noise levels in the habitat: at increasing SPL, individuals consistently reduced their time absent after release from the nest area.

In contrast, under short-term boat noise playback conditions, there was no change in the time a goby was absent after predator release. This lack of change was caused partly by individuals that did not leave the site after the predator strike and partly by individuals that were already absent part of the time before the predator strike: absence in the period before the predator strike was significantly more likely to occur during boat noise playback than during ambient noise playback (Fig. 6; Table 1). When controlling for time absent before the predator strike by taking only individuals that did not leave

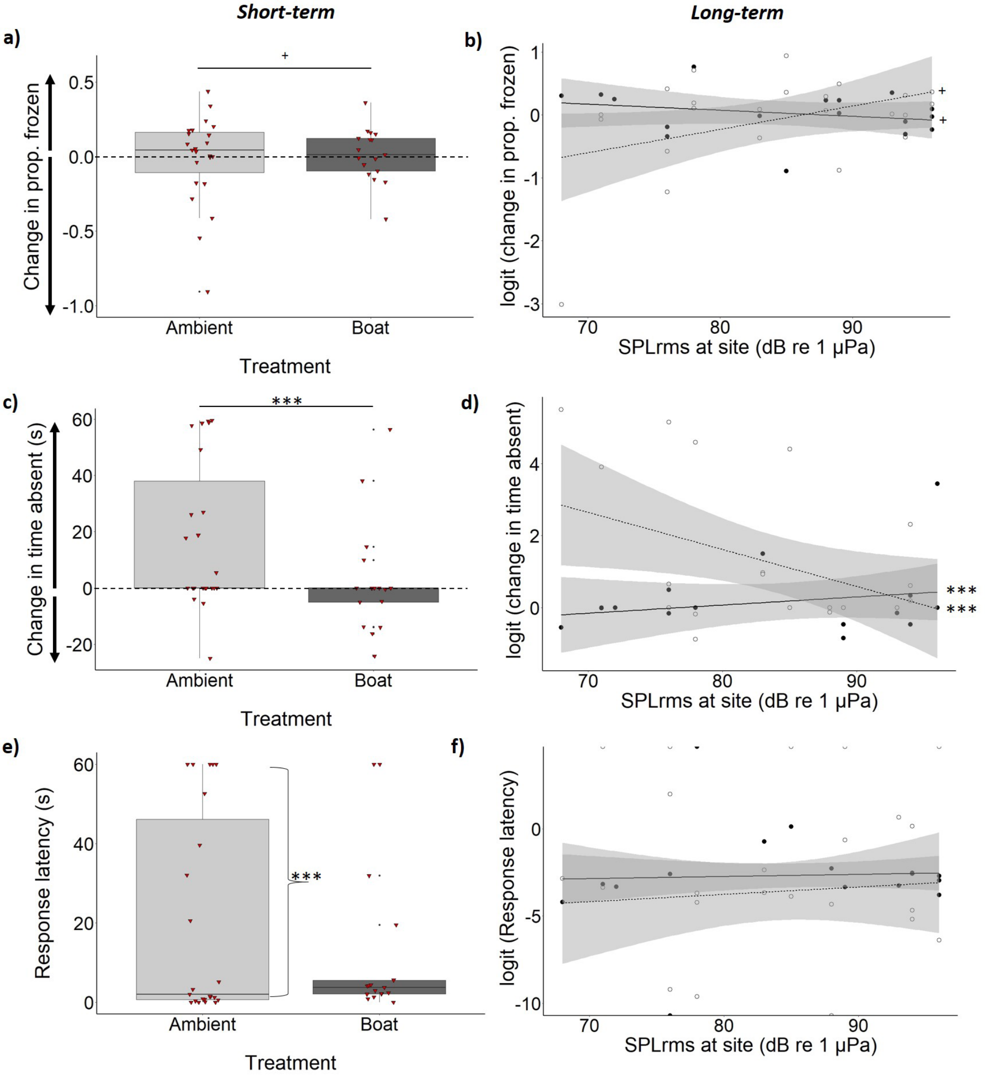

Figure 5. Effect of short-term ambient noise (light grey, N = 23) and boat noise (dark grey, N = 17) playback on a, b) the change in the time gobies were frozen before and after predator release, c, d) the change in time gobies were absent before and after predator release, and e, f) the response latency to the simulated predator. b), d) and f) show correlations during playback of ambient noise (dashed line, open circles) and boat noise (solid line, closed circles) between long-term ambient noise levels at the experimental site and the logit-transformed b) change in time gobies were frozen, d) change in time gobies were absent, and f) response latency to the simulated predator. Significant differences and correlations are indicated with asterisks (***), while non-significant trends (p<0.1) are indicated with a plus (+). Note that the average response latency did not change significantly between ambient and boat noise playback, but rather the variance changed significantly (accolade with asterisks).

the site, all factors were still significant (Table 2). The lack of change in absence was not influenced by the longer-term noise levels of the habitat: it was irrespective of SPL (fig. 5d). At the highest habitat long-term noise levels, responses to short-term ambient noise mirrored those under short-term boat noise, with no increase in time absent.

### Response latency

Gobies did not significantly change their response latency to predator release under boat noise playback compared to ambient noise playback (Fig. 5e, Table 1). However, the strong variation in individual response observed in short-term ambient noise conditions disappeared nearly entirely under boat noise playback conditions. Gobies under boat noise playback showed a significantly smaller variation in response latency (Precision value: 95% CI estimate range = 0.51-2.83). Gobies reacted significantly faster to the predator when they startled, both during boat and ambient noise playback (Table 1). Ambient SPL at the location did not influence response latency for either exposure

Table 2: For gobies that were present 100% of the observed time before predator release, the time they were absent after predator release was influenced by playback type and SPL. Variables that significantly influenced the observed time absent are indicated with an asterisk (*).

| | **Estimate** | **SE** | **z** | **p** |
|---|---|---|---|---|
| Intercept* | 8.72 | 2.39 | 3.65 | <0.001 |
| Playback type (boat noise)* | -12.13 | 3.78 | -3.21 | <0.01 |
| SPL* | -0.09 | 0.028 | -3.32 | <0.001 |
| Playback type:SPL* | 0.14 | 0.044 | 3.12 | <0.01 |
| *Precision (φ)* | | | | |
| Intercept* | 1.38 | 0.33 | 4.16 | <0.0001 |
| Playback type (boat noise) | 0.35 | 0.60 | 0.59 | 0.6 |

context (Fig. 5f).

## Discussion

Our results revealed that both long term and short-term noise levels significantly altered the anti-predator response of free-ranging marine prey. Under natural conditions, simulated predator presence invoked an avoidance response in gobies, making them leave the direct area of their nest. However, the experimental predator attack-induced displacement depended on both short- and longer-term noise levels of the habitat. Short-term boat noise induced a lower change in absence in response to the predator: individuals left the site for shorter periods with boat noise playback than with ambient noise playback. This effect was independent of longer-term noise levels in the habitat. Individuals exposed to high levels of long-term noise, however, also showed suppressed anti-predator flight response under ambient noise playback conditions. The higher the longer-term noise levels, the more similar anti-predator behaviour of the gobies under short-term ambient noise was to that of gobies under short-term boat noise. Hence, short- and long-term noise levels in their marine habitat interacted to modulate the goby anti-predator response.

### *Short-term effect of boat noise*

Our results provide a clear example of impact from boat noise on the response strength to the visual stimulus of a looming, simulated predator. The playback of boat noise made gobies be absent for shorter periods, while they also tended to freeze less. This occurred without an apparent delay in detection or processing of the stimulus, as reflected by the lack of a significant effect on response latency. Hence, the hypothesis that the sand gobies would be distracted by noise does not hold.

Several earlier studies with a similar approach have found effects that did fit an explanation in the context of distraction (Chan and Blumstein 2011). The extra sensory input of the sound could lead to less processing capacity for the risk assessment of the visual predator stimulus. However, other experimental studies that reported a sound-related change in anti-predator behaviour that was explained by distraction all showed a larger response latency for distracted animals (Chan et al. 2010; Simpson et al. 2015; Morris-Drake et al. 2016; Simpson et al. 2016; McCormick et al. 2018). Although the variance in response latency did decrease, gobies did not change their average response latency to the predator stimulus under boat noise playback. This suggests that the gobies in this study were not distracted.

It is likely that the addition of the sound stimulus used in this study decreased the vigilance levels of the sand gobies in response to the visual stimulus (Frid and Dill 2002), possibly in a form of cross-modal habituation (Sörqvist et al. 2012; Halfwerk and Slabbekoorn 2015). Many species are known to show a varying level of vigilance behaviour that can be related to cues in their environment that are associated with smaller or larger predation risk (Laundré et al. 2010, Magrath et al. 2015). The acoustic cue of a boat could be associated with non-predatory presence of humans in the water, leading to a lower risk-level of overpassing shadows. The observed decrease in variation in response latency could point to this, as it might indicate less ambiguity in the risk determination of the (to the gobies) unknown moving predator stimulus. Alternatively, it could be that gobies exposed to boat noise playback were already in a state of heightened vigilance, which would lead to a smaller change in behaviour after the predator strike. In this case, the gobies exposed to boat noise should already be absent part of the time before predator release. Indeed, when examining which gobies were temporarily absent in the period before predator release, nearly all of these were gobies exposed to playback of boat noise.

### *Site-dependent long-term effect*

Combining the effects of short-term noise playback with existing long-term noise conditions showed that long-term exposure to noise changed the baseline response of gobies to a simulated predator. Gobies living in long-term noisy sites and exposed to playback of ambient noise tended to freeze for shorter periods when exposed to the simulated predator and did not increase their time absent from the site. Furthermore, while gobies living in quiet sites that were exposed to boat noise temporarily left their nests even before the predator strike, gobies in noisy sites seemed to do so less (Fig. 6), although the sample size was too small to be conclusive. After the predator strike, there was no change in time absent for gobies exposed to boat noise playback, regardless of the noisiness of the site. Hence, when exposed to boat noise, gobies in noisy sites likely stayed put after predator release, while gobies in quiet sites kept leaving the site, as they had done before release.

The increased time absent before predator release suggests that boat noise playback induced a heightened vigilance state in sand gobies, but that this effect diminished with site noisiness. Hence, gobies that were frequently exposed to boat noise may have had higher tolerance levels than gobies from quiet sites. Animals exposed to long-term disturbance often have increased tolerance to short-term disturbance (Bejder et al. 2009). For example, wild cichlids from sites with relatively high acoustic disturbance did not significantly increase their oxygen consumption levels when exposed to short-term playback of boat noise, while their conspecifics from lower-disturbance sites did (Harding et al. 2018). In a semi-open experiment, Nedelec et al., (2016) showed that a coral reef fish increased its hiding behaviour in response to boat noise when first exposed, while this response was absent after a week and a half of repeated exposure.

The varying levels of vigilance induced by the boat noise playback put the lack of behavioural change upon predator release into perspective. Even though gobies from all sites seemed to respond similarly when exposed to a predator strike under boat noise playback, the pre-existing differences in time absent suggest that gobies in quiet sites had an increased vigilance state to which the predator strike added little. In contrast, the gobies in noisy sites may have had a diminished vigilance state, that led to a reduced response to the predator strike, regardless of the type of sound exposure.

In terrestrial settings, animals living in areas that are more frequently disturbed by humans often show diminished anti-predator behaviour (Lowry et al. 2013). This is hypothesized to have two possible causes: 1) habituation to non-threatening stimuli that leads to overall reduced responses to disturbance; 2) reduced exposure to predators that avoid disturbed sites (Geffroy et al. 2015). Perhaps our noisy sites contained fewer predators, as (especially larger, marine mammal) predators often avoid acoustic disturbance when the site is not vital for their survival (Carstensen et al. 2006; Kok et al. 2018). However, the relatively short distances between the sites make this less likely. If actual predation levels were not diminished, gobies in long-term noisy conditions would suffer an increased risk of predation in these sites.

### *Conclusions*

Both short-term and long-term disturbance by boat noise affected the anti-predator behaviour of free-ranging sand gobies in this study. Even though it is not clear whether these changes will have detrimental consequences for the gobies themselves, it is important to realise that not only pristine (in terms of acoustic pollution) areas might be affected by noise disturbance and would require conservation effort. Decreasing the noise disturbance in areas with more long-term acoustic pollution should also be considered, since our results show that long-term disturbance can lead to behavioural changes beyond the direct period of noise exposure (i.e. in ambient conditions). Since both the behaviour of the predator and the prey affects the outcome of the interaction (DiRienzo et al. 2013), a change in behaviour in one of the two is likely to result in changes for both parties. Documenting not only short-term but also long-term effects of noise on animals, preferably at community level, will give most insight into the possible ecosystem changes that may occur as a result of noise disturbance.

## Acknowledgments

The authors would like to thank all people assisting in the field work, as well as boat owners that made their vessel available for recordings. The project was licensed by the Dutch Ministry for Infrastructure and the Environment (Rijkswaterstaat; project number RWSZ2018-00003561B).

# 7

# General discussion and summary

The effects of anthropogenic noise on interactions between predators and their prey are still little understood. This thesis aims to fill pressing knowledge gaps on this topic by studying how anthropogenic noise affected various stages in the prey-hunting of predators and the predator-avoidance of prey. For predators, I investigated whether anthropogenic noise could influence habitat choice (chapters 2 and 3), foraging efficiency (chapter 3) and communication between foraging group members (chapter 4). For prey, I studied how anthropogenic noise affected prey behaviour outside of predation contexts (chapter 5) and if current and previous vessel noise exposure affected anti-predator behaviour when a simulated predator attacked (chapter 6). Next to novel insights, my results provide a proof of concept and point to promising avenues of further research on this recently emerging field. Below, I will discuss the conclusions from the various chapters and will indicate the implications for conservation of marine communities in the oceans. Finally, I will discuss what further research is needed to further improve our understanding of the effect of anthropogenic noise on predator-prey relationships.

## *The effects of sound on predators*

As shown in chapters 2 and 3, an increase in ambient noise conditions can induce avoidance of a preferred area by harbour porpoises (*Phocoena phocoena*). The results of this study do not only corroborate earlier observations of harbour porpoises leaving noise-disturbed areas, such as pile-driving construction sites (Carstensen et al. 2006; Tougaard et al. 2009; Dähne et al. 2013), but also match studies on other marine mammals: Blainville's beaked whales (*Mesoplodon densirostris*), for example, stopped prey-search and likely avoided areas with sonar exercises (McCarthy et al. 2011). Nevertheless, whether or not animals will avoid an area, as well as the extent of avoidance, will be influenced by more than just the presence of sound. If the area is the only suitable foraging habitat in the surrounding waters, animals will have limited capacity to avoid it completely, as was observed for Cuvier's beaked whales (*Ziphius cavirostris*) in a Navy training range (Southall et al. 2019).

If predators decide to stay and forage in a noisy area, they may face consequences on foraging efficiency. Zebrafish (*Danio rerio*) preying on water fleas made more handling errors when catching prey under noisy conditions than under ambient conditions (Shafiei Sabet et al. 2015). In contrast, the harbour porpoises in my study (chapter 3) did not show a decreased performance in prey searching. This could be due to the set-up of the experiment, which is likely to have been easier than searching for prey in a natural context. Interestingly, a study that showed that Ambon damselfish (P*omacentrus amboinensis*) were more likely to be predated in noisy conditions, implicitly also showed that their predator was not detrimentally affected by the noise in its foraging performance (Simpson et al. 2016). Predators caught the damselfish with fewer attempts under boat noise playback than under ambient noise playback. This could be explained by a change in behaviour of the damselfish, as they were less likely to startle to a simulated predator attack. Whether anthropogenic noise influences foraging efficiency of predators therefore not only depends on the direct impact on the predator, but also the indirect impact on the behaviour of the prey.

For group-living predators, communication during and after foraging may be affected by increased ambient noise levels. For long-finned pilot whales (*Globicephala melas*), acoustic communication after a deep foraging dive is a potential mechanism for relocating group members (chapter 4). Ambient noise levels affected call detectability, but calls produced in a noisier environment were also louder and longer, possibly to cope with the noise. In bottlenose dolphins (*Tursiops truncatus*), calls produced in low-frequency noisy conditions were found to be of higher frequency and with fewer changes in frequency (Heiler et al. 2016; Fouda et al. 2018; van Ginkel et al. 2018). These changes in call characteristics are hypothesized to counteract decreases in the range of call detectability, and are similar to changes found for vocalisations in terrestrial taxa (Halfwerk and Slabbekoorn 2009; Luther and Gentry 2013). Also, for the terrestrial species, it remains to be proven that the benefits of masking avoidance outweigh the potentially detrimental consequences of signal modification in terms of energy budget and compromised signal value (Slabbekoorn 2013; Read et al. 2014).

## *The effects of sound on predator-avoiding prey*

Anthropogenic noise may induce or elevate the level of vigilance in prey animals, because it is perceived as a risk (Frid and Dill 2002). Schooling fish around wind farms increased their cohesion and swam higher in the water column in relation to exposure to a seismic survey and pile driving activity. Nevertheless, response type and strength were within the natural behavioural variation observed outside the exposure period (chapter 5). Due to the small sample size of this study, no general conclusions should be taken from the patterns found. However, both parameters have been found to change in other studies where fish were exposed to noise, albeit that most fish dive down instead of swim up in the water column (Doksæter et al. 2012; Fewtrell and McCauley 2012; Hawkins et al. 2014b; Neo et al. 2014; Neo et al. 2015a). Future studies should strive for replicated experimental design to be able to assess causal relationships between seismic noise and prey behaviour and how resulting changes in the prey field would affect foraging predators.

When a predator attacks a prey under noisy conditions, the prey might have an altered perception of risk and thereby change the degree of its anti-predator response (Frid and Dill 2002). In chapter 6, I investigated how long-term exposure to boat noise influenced the anti-predator behaviour of sand gobies (*Pomatoschistus minutus*) under ambient and short-term boat noise exposure conditions. Sand gobies were less likely to leave the site of the predator attack under playback of boat noise than under playback of ambient noise. Hence, short-term boat noise suppressed the goby avoidance response. This effect became more pronounced in noisy habitats, where gobies exposed to ambient noise also stayed on site after a predator attack. The decreased perception of risk may be detrimental for the prey, if the foraging performance of the predator does not diminish. However, since the predator is often also affected by the noise, general predictions for the impact of anthropogenic noise require research on the community level.

### Community effects

Sand gobies are a common prey species of harbour porpoises (Leopold 2015). The cryptic defence strategy of gobies is ineffective against harbour porpoises, which are acoustic (active echolocation) predators (Magnhagen and Forsgren 1991; DeRuiter et al. 2009). Since sand gobies are predicted to be less likely to flee away from an attacking predator under boat noise conditions (chapter 6), harbour porpoises are likely to have more of an advantage when ambient noise levels are higher. This is strengthened by the lack of effect of noise found on prey-searching behaviour of harbour porpoises (chapter 3). Although a subsequent study with the same individuals on prey-catching behaviour during exposure to pile-driving noise showed increased termination of prey-catching attempts under high noise levels of one individual (Kastelein et al. 2019), gobies may respond differently to this sound source. Nevertheless, the benefit for harbour porpoises under noisy conditions only holds true for those porpoises that will stay in noisy areas, which they will likely tend to avoid (c.f. chapter 3). Thus, the resulting predator-prey dynamics will become more complex, as noise adds one more layer to the existing layers of behavioural trade-offs, physical conditions and species distributions (Gaynor et al. 2019).

Pilot whales hunting deep-water prey that are exposed to sonar can stop foraging (Miller et al. 2012). However, the duration of the response was unknown and not all animals responded in the same manner. Supposing pilot whales would not stop their foraging bout, would there be changes in their hunting efficiency due to changes in prey behaviour? Both pelagic fish and squid can be behaviourally affected by sound (chapter 5, Doksæter et al., 2012; Hawkins et al., 2014b; Mooney et al., 2016), but effects of sound on anti-predator behaviour of either taxon have not been studied. Exposure to sound itself induced increased school cohesion and deeper swimming in fish and jetting and inking in squid. These behaviours are also seen in response to predator attacks, so could be regarded as escape or at least increased vigilance (Malavasi et al. 2004; Langridge 2009; Rieucau et al. 2014). However, whether these behavioural changes will be beneficial for the prey depends on timing in relation to foraging bouts of the pilot whales, duration of the change, which type of anthropogenic noise induces the changes and how the pilot whales are affected by that same noise in their foraging performance. Future studies on foraging behaviour of free-ranging pilot whales under noisy conditions can provide insight and may be conducted using suction-cup tags for the whales, combined with a visualisation method for the prey.

### Implications for conservation

In recent years, there has been increasing realisation that effects of anthropogenic noise in the ocean should be mitigated. The European Union has added increased ambient noise levels as a pollutant that should be addressed to achieve Good Environmental Status in European waters (Dekeling et al. 2016). Non-governmental organisations such as WWF have asked attention for the effects of anthropogenic noise on marine life (https://www.wwf.nl/kom-in-actie/arctic-noise-petitie) and legislation is in place that requires noise mitigation efforts to take place during construction of wind farms and other offshore activities in national waters (2012). At the same time, mitigation efforts can only be successfully designed and implemented under scientific support.

The current mitigation measures that are being applied mostly focus on decreasing the received level of noise by animals in the vicinity of human activity. Examples of this are bubble curtains around piles that are being driven into the ground that block the sound emitted from the hammer strikes and ramp-up procedures before the start of noisy activities to alert and deter animals from the area. The concept behind these measures is that lower noise levels will have a lower impact on the marine community than higher noise levels (Gomez et al. 2016). Although higher noise levels can induce effects beyond altered behaviour (e.g. hearing threshold shifts, physical damage), the large spatial scale of lower noise levels will affect a lot more individuals and behavioural changes due to low levels of noise can have a similar fitness impact (Slabbekoorn et al. 2010; Popper 2012).

In relation to science-guided mitigation and conservation efforts, my results indicate that conservation should not only focus on protecting or creating pristine environments in terms of noise, but also include more noise-polluted areas. Animals living in noise-polluted areas may show changes in behavioural strategies even when the noise has ceased, which could be detrimental to their survival. For example, long-term increased levels of ambient noise in sand goby habitats led to changes in anti-predator behaviour even under quiet conditions (chapter 6). This indicates that sand gobies in noise-polluted areas did not have altered tolerance levels to the disturbance, but rather had a permanently different behavioural strategy than gobies living in quiet habitats.

Other studies did show that animals increased their tolerance to longer-term noise exposure, either by going back to baseline behaviour during exposure (Neo et al. 2018) or by showing no change in physiology when the noise started after a period of quiet (Harding et al. 2018). However, my study signifies that this will not be the case for every species. Moreover, as sound attenuates little in water, finding entirely noise-pollution-free environments will be difficult. Creating protected marine areas that exclude human activity can, however, improve the ambient noise levels in the centre of that area (Herrera-Montes 2018). Besides that, technological innovation can reduce noise pollution levels, as the quieting of ship engines over the past years has already led to a stagnation in ambient noise level rises in some areas of the world (Miksis-Olds and Nichols 2016).

Additionally, when deciding which areas to designate as nature reserves, focus on those areas that are preferred foraging spots of predators. Predators, especially apex predators, often control the dynamics of the ecosystem and their disappearance can lead to trophic cascades (Estes et al. 2011). Even if ambient noise levels are high, some predators might stay in those areas, if alternative habitats are not available or of much lower quality. However, this might have possible detrimental consequences for foraging efficiency. For example, foraging Cuvier's beaked whales stayed in a naval training area despite naval exercises, which was also the area with the highest prey abundance in the vicinity (Southall et al. 2019), but the same species is also known to stop foraging in response to sonar (DeRuiter et al. 2013; Falcone et al. 2017). Moreover, foraging spots are usually preferred because of a high abundance of prey, so protecting those areas will also lead to protection of more prey animals (Wirsing et al. 2010).

### *Current knowledge gaps and suggestions for further research*

In this thesis, I have described several possible effects of anthropogenic noise on both predator and prey, in various stages of the predator-prey interaction. My results clearly indicate that anthropogenic noise may impact predator-prey dynamics. However, for effective conservation measures we need to understand how these impacts translate into effects on the ecosystem. Therefore, further research is needed to elucidate how these changing dynamics may alter the balance between predator and prey population and how it translates to effects on food web dynamics.

It is important to investigate the mechanisms that underlie the effect of noise on behaviour of predator and prey, so it will become possible to predict how species outside of the study system will be affected by noise (Slabbekoorn et al. 2018). For instance, what mechanism underlies if a prey species will be distracted or become more vigilant due to the noise (Chan and Blumstein 2011; Voellmy et al. 2014b)? It could be related to the association of the presented noise to a predatory threat, but if so, would that change with experience? Even long-term exposure does not always lead to increased tolerance levels (chapter 6). Understanding mechanisms like these are needed to extrapolate the effect of noise on one predator-prey combination to other possible predator-prey combinations of the two study species. Only then can we start to paint a picture of how the ecosystem is affected.

Another topic that deserves further investigation is the effect of long-term exposure to noise. The results of chapter 6 show that habituation does not always occur when animals are exposed to noise over longer periods. Are there indicative parameters that could predict how animals will react in the long-term? And is it possible to reverse changes in behaviour that are induced by long-term exposure to noise? Answers to these questions are necessary to understand fundamental behavioural processes, but also to be able to guide conservation efforts. If behavioural effects of long-term exposure to noise turn out to be non-reversible, this might change how conservation efforts should be focused.

These and other questions should be answered through a combination of theoretical modelling, experiments in captivity and field studies (Slabbekoorn et al. 2018). Only when combining these techniques will it be possible to really understand all aspects of the problem. The study on captive harbour porpoises in chapters 2 and 3 showed that it is possible to provide a proof of concept of the mechanisms underlying behavioural responses seen in the wild. The study on free-ranging pilot whales in chapter 4 proved that combining theoretical modelling with field observations can lead to insights that are otherwise difficult to acquire. The results of the study on pelagic and benthic fish in chapters 5 and 6 stressed the need for thorough replication in field studies, as the response of free-ranging animals most closely approaches how individuals will respond in their natural environment. And finally, the experimental field study of chapter 6 showed that when an easily observable study organism is chosen, a field experiment can produce high sample size data sets on individuals in their own environment – and thus provide information on how individuals will actually respond.

## *Conclusion*

The multi-disciplinary studies of this thesis combine to unravel more insight into the influence of noise on predator-prey relationships in the marine environment. This thesis showed that: 1) harbour porpoise swimming speed and surfacing rate can be used as independent metrics to analyse porpoise spatial behaviour (chapter 2); 2) two captive harbour porpoises avoided noisy areas, but when forced to search for prey in noise, did not lower their search performance (chapter 3); 3) long-finned pilot whales that were separated vertically when part of the group is foraging had the potential to keep in contact through acoustic communication, but fewer calls were detected in higher ambient noise levels at the receiver (chapter 5); 4) sound from a seismic survey and from pile driving could potentially disturb spatial behaviour of pelagic fish schools at wind farms in the North Sea; 5) exposure to long-term acoustic disturbance by boat noise interacts with the effect of short-term boat noise playback on the anti-predator behaviour of sand gobies.

Further investigations should focus on revealing the mechanistic underpinning of noise effects on behaviour of both predator and prey. However, effective noise mitigation measures will not only depend on thorough knowledge of the impacts but also on economic and political interests. Trade-offs exist, for instance, with the still-increasing demand for green energy. For conservation measures to be effective, therefore, collaboration between stake holders is necessary, preferably on an international scale. Noise does not respect nation boundaries and changes that could have a large impact, such as silencing ship engines, need to be applied by many countries before any improvement will be visible. To properly conserve marine ecosystems from noise, we do not only need to study the effects on a higher scale of species interactions, but we should also zoom out when considering mitigation measures to find out if they are not counteracted by other processes affecting the animals. Only then will we be able to reduce the impact of noise on marine ecosystems.

# Nederlandse samenvatting

## Het lawaai van de jacht: effecten van lawaai op predator-prooirelaties in een marien ecosysteem

Bijna alle dieren die in het water leven zijn gevoelig voor geluid. Dieren gebruiken geluid om zich te oriënteren, om predatoren te vermijden of prooi te zoeken, maar ook om partners te lokken of concurrenten te verjagen. Sinds het begin van de Industriële Revolutie is het geluidsniveau in de zeeën toegenomen door menselijke activiteiten, zoals scheepvaart en heien. Dit toegenomen geluidsniveau verstoort het zeeleven. Dieren kunnen fysieke schade oplopen als ze erg dicht bij een luide geluidsbron zijn, zoals gehoorschade of een gescheurde zwemblaas (bij vissen). Hierdoor kunnen een klein aantal dieren in een beperkte zone zelfs fataal beschadigd raken. Met meer achtergrondgeluid vertonen dieren ook gedragsveranderingen, zoals het vermijden van lawaaierige gebieden. Deze gedragsveranderingen kunnen al bij een kleine toename van het geluidsniveau optreden, met als consequentie dat een groot aantal dieren in een groot gebied met geluidverstoring hiermee te maken krijgt.

Een van de gedragsveranderingen die directe gevolgen voor het leven van dieren kan hebben, is die op interactie tussen predator en prooi. Predatoren moeten efficiënt jagen om genoeg te kunnen eten, terwijl het voor hun prooi van belang is niet opgegeten te worden. Door verhoogde geluidsniveaus kan de predator-prooirelatie veranderen: predatoren zouden bijvoorbeeld lawaaierige gebieden met prooi kunnen gaan vermijden, of zouden afgeleid kunnen raken door het geluid tijdens het foerageren. Tegelijkertijd zou hun prooi directe verstoring van het geluid kunnen ondervinden, of afgeleid kunnen raken als ze in lawaaierige omstandigheden worden aangevallen door een predator. Het begrijpen van de veranderingen die optreden tijdens predator-prooi-interacties kan ons helpen om te begrijpen welke ecosysteemveranderingen er kunnen optreden door menselijke geluiden. Er is echter nog nauwelijks onderzoek naar dit onderwerp gedaan.

In mijn proefschrift heb ik verschillende aspecten van de predator-prooi-interactie onderzocht, om zo een beter begrip te krijgen van hoe predator-prooirelaties veranderen onder invloed van lawaai. Bij de predator heb ik gekeken naar mogelijke verstoringen voor, tijdens en na het foerageren. In hoofdstuk 2 en 3 laat ik zien dat bruinvissen in gevangenschap een voorkeursgebied gingen vermijden als dat lawaaierig werd gemaakt. Dit suggereert dat bruinvissen in een verstoorde omgeving mogelijk een kleiner jachtgebied hebben. Een predator zal echter niet altijd de kans hebben een lawaaierige omgeving te vermijden. Daarom heb ik in hoofdstuk 3 ook onderzocht of bruinvissen meer moeite hebben met het vinden van een prooi in lawaaierige omstandigheden door middel van een prooi-zoek-test met drie mogelijke prooilocaties. De bruinvissen in dit onderzoek waren net zo efficiënt in het vinden van de prooi met en zonder lawaai, wat suggereert dat foeragerende bruinvissen minder snel verstoort raken bij dit onderdeel van de jacht, voor de spectra en niveaus die we getest hebben.

Voor predatoren die in groepen leven maar individueel jagen, is het van belang andere leden van de groep terug te kunnen vinden als de jacht voorbij is. In hoofdstuk 4 laat ik zien dat grienden met behulp van geluidsignalen elkaar zouden kunnen detecteren, met zowel data van gezenderde dieren als theoretische modellen van geluidspropagatie. Door te kijken naar hoe karakteristieken van de omgeving en de geluiden zelf samenvallen met de detectie van de geluiden, laat ik zien dat het niveau van achtergrondgeluid een belangrijke voorspeller is voor de mogelijke detectie van een geluid. Dit impliceert dat een verhoging van het achtergrondgeluid zou kunnen leiden tot een lagere kans op detectie van de geluiden van soortgenoten. Voor sociaal levende dieren kan dit grote gevolgen hebben voor het terugvinden van de groep, wat tot ongunstig energieverbruik en verhoogd predatierisico kan leiden.

De dynamiek van de relatie tussen predator en prooi wordt niet alleen bepaald door de predator, maar ook door de prooi. Daarom heb ik gekeken of prooivissen hun gedrag veranderen onder invloed van geluid buiten een predatiecontext en tijdens een aanval van een predator. In hoofdstuk 5 heb ik gekeken naar het ruimtelijke gedrag van pelagische visscholen in windmolenparken met drie verschillende geluidcontexten: tijdens een seismisch onderzoek, tijdens het heien voor een nabijgelegen windmolenpark, en tijdens een controlesituatie zonder specifieke geluidsblootstelling. Tijdens het seismisch onderzoek waren er minder visscholen die dichter bij elkaar zwommen. Ook tijdens het heien zwommen de visscholen dichter bij elkaar en hoger in de waterkolom. Er waren echter ook significante veranderingen in het gedrag van visscholen die niet aan geluid waren blootgesteld. Dit impliceert dat we voorzichtig moeten zijn met de conclusies die we uit deze studie trekken, wat betreft de zekerheid over het oorzakelijke verband. Herhaling van observaties is nodig voor meer verstrekkende conclusies.

Van direct belang voor de overleving voor een prooidier is zijn reactie op een aanvallende predator. In hoofdstuk 6 heb ik dikkopjes (een belangrijke prooivis van bruinvissen) blootgesteld aan een gesimuleerde aanval van een predator met en zonder bootgeluid. Onder invloed van bootgeluid verlieten de dikkopjes het gebied minder vaak als de gesimuleerde predator aanviel. Daarnaast heb ik het niveau van geluidsverstoring van de verschillende leefgebieden van de dikkopjes gerelateerd aan hun reactie op de predatoraanval. Dikkopjes die in een gebied met veel verstoring leefden, waren ook in situaties zonder bootgeluid minder geneigd om weg te schieten in reactie op de aanval van de predator (vergeleken met dikkopjes op relatief onverstoorde plekken). De resultaten laten zien dat langdurige verstoring door geluid voor gedragsveranderingen kan zorgen die niet alleen tijdens de verstoring optreden, maar ook als er even geen verstoring is.

De resultaten van dit proefschrift laten zien dat verschillende aspecten van de predator-prooirelatie veranderen door geluidverstoring. Veranderingen die nadelig zijn voor de ene partij, kunnen voordelig zijn voor de andere partij. Zo kan het feit dat dikkopjes minder sterk reageren op een aanval van een predator tijdens bootgeluid ervoor zorgen dat bruinvissen makkelijker kunnen foerageren. Tegelijkertijd zijn de uiteindelijke consequenties van geluidverstoring juist door deze interacties tussen predator en prooi vaak moeilijk te voorspellen. Dit proefschrift is dan ook maar een eerste stap in het opvullen van deze kennislacune. Vervolgonderzoek naar de mechanismen die aan de gedragsveranderingen ten grondslag liggen, evenals het beter begrijpen van lange-termijn effecten van geluidverstoring, is noodzakelijk om te weten hoe de balans van mariene ecosystemen zal veranderen onder invloed van menselijk geluid. Alleen met deze informatie kunnen we de effecten van menselijk geluid op mariene ecosystemen begrijpen en tegengaan.

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# Acknowledgments

I have heard people say that doing a PhD can be a lonely job, but I have never felt like I was on my own. First of all, I could always turn to my supervisors, Hans, Fleur and Carel, who challenged me to always get the most out of myself and who supported me when life got in the way. The Behavioural Biology group that I have been part of was the most warm and caring group I could have wished for. I especially want to thank Fabian, Inge, James, Jeroen, Jing, Judith, Merel, Michelle, Peter and Temp. They knew how to brighten my workday with a laugh and always pushed aside their own busy schedule to help me out if I needed it. Another big thank-you goes to Sander for explaining and re-explaining acoustics to me.

A PhD-project can be all-consuming at times and requires you to push yourself to the edge. Thank you, Alex, for making sure I never fell over that edge and for making me feel loved these past four years. Other people that I could turn to were my friends. Judith, thank you for listening to my self-pity stories, for all the Friday-night dinners together with Sjoerd that helped me relax after a busy week and of course for being my paranymph. Also, a wholehearted thanks to my other paranymph, Irene, who is always there for me despite living on the other side of the country. Nadine, thank you for your enthusiasm that even extended to helping me out with the field work several times. Saskia, thank you for all the good talks we have had and for the insider PhD-life advice.

Halfway through my PhD-project I was crazy enough to decide that explaining my PhD-topic through dance would be a good idea. Thank you to Aafke, Judith, and all other dancers for supporting me in my craziness and helping me make an amazing video that can educate laymen about my work.

Finally, I would like to thank my parents and my brother and sister. Not only did you stand by me during these years as you always do, you also helped me out with various aspects of my project. I can safely say that without your helping hands, my 2018 field season would not have been successful. I feel fortunate to have you as my family and look forward to future family-fieldwork!

# Curriculum Vitae

Annebelle Kok was born in 1989 in Leiden, the Netherlands. She attended school at Het Rijnlands Lyceum Oegstgeest from 2001 to 2007, following a bilingual VWO-Gymnasium programme. In her penultimate school year, she followed the pre-university course LAPP-Top, focussing on Comparative Indo-European Linguistics. After school, she obtained her Bachelor's degree in Biology at Leiden University between 2007 and 2010, followed by a Master's degree in Evolution, Biodiversity and Conservation between 2010 and 2013, also at Leiden University.

During her Bachelor, she did an internship with Dr Caroline van Heijningen and Prof Dr Carel ten Cate, setting up a protocol for a habituation/dishabituation paradigm in zebra finches. In the final year of her Bachelor, she also participated in an honours programme focussing on better understanding photosynthesis to optimize solar energy techniques. During her Master, she did two internships, one with Dr Liew Thor-Seng and Prof Dr Menno Schilthuizen, working on shell growth and development in two snail species. Her second internship was with Dr Fleur Visser and Dr Hans Slabbekoorn, where she investigated the relationship between social, vocal and diving behaviour in long-finned pilot whales.

After graduating, she worked as a self-employed scientist from 2013 to 2015, spending the summers of 2013 and 2014 as a marine mammal field scientist and data analyst at Kelp Marine Research. In the summer of 2015, she started her PhD on the effects of noise on predator-prey relationships in a marine ecosystem, which she finished at the end of 2019. During her PhD, she conducted several scientific studies, as well as spending one-third of her time teaching Bachelor and Master students. She received several awards for communicating science through talks or articles. Currently, she is starting a Postdoctoral position at Scripps Institution of Oceanography in La Jolla, United States.

# Publications

Liew, T.-S., **Kok, A. C. M.**, Schilthuizen, M., & Urdy, S. (2014). On growth and form of irregular coiled-shell of a terrestrial snail: Plectostoma concinnum (Fulton, 1901) (Mollusca: Caenogastropoda: Diplommatinidae). PeerJ, 2, e383. https://doi.org/10.7717/peerj.383

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