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

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

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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'

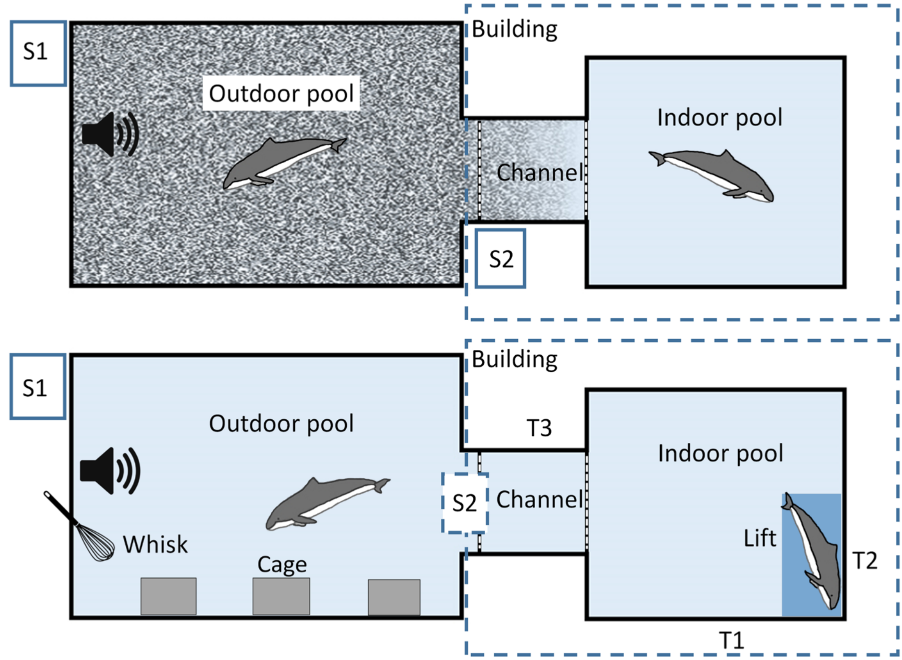


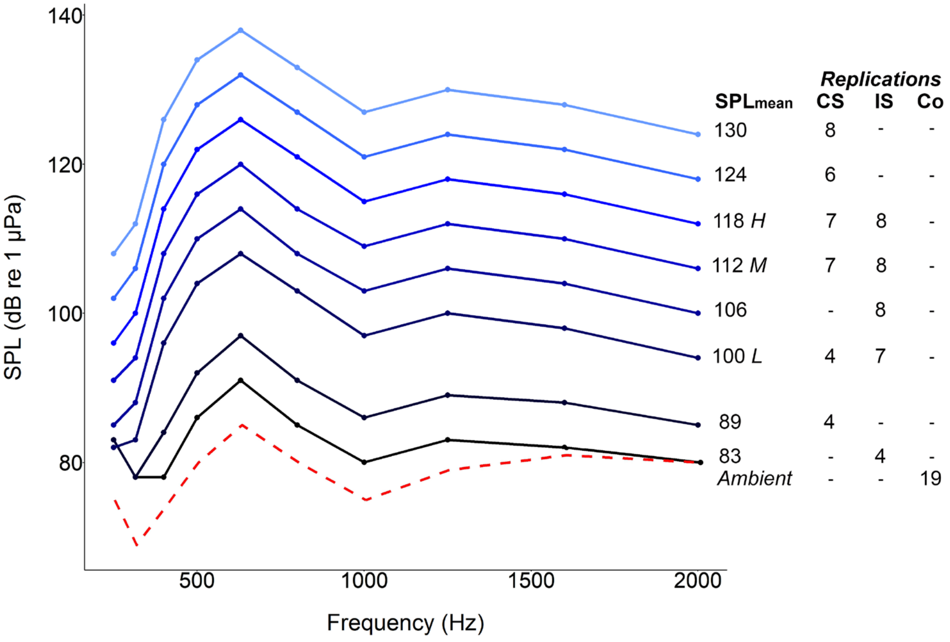
Figure 1: Top-view of the setup for experiment 1 (top) and 2 (bottom). S1 = scientist 1, S2 = scientist 2, T1 = trainer 1, T2 = trainer 2, T3 = trainer 3. Black/white shaded area in top left pool indicates the area with elevated sound levels. Interrupted black and white lines to the left and right of 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



SPLmean	Replications CS	IS	Co
130	8	-	-
124	6	-	-
118 *H*	7	8	-
112 *M*	7	8	-
106	-	8	-
100 *L*	4	7	-
89	4	-	-
83	-	4	-
Ambient	-	-	19

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).

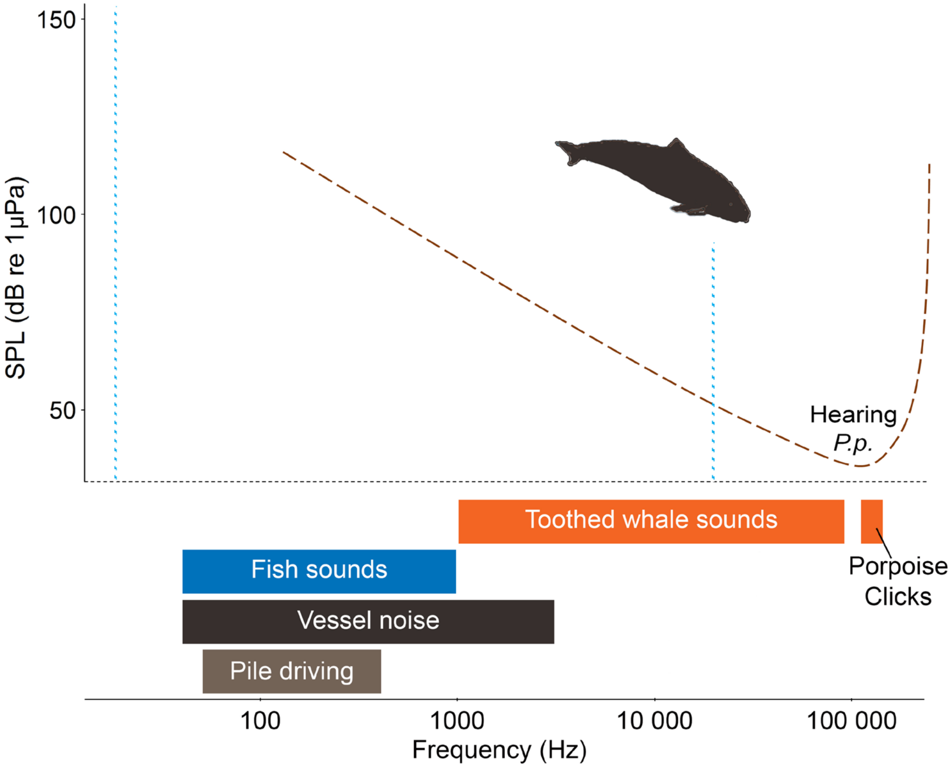


Figure 3: Schematic representation of general frequency bandwidths of pile driving sound (brown-grey; Göttsche et al., 2015; Schecklman et al., 2015), ship sound (black; McKenna et al., 2012; Simard et al., 2016), sounds produced by fishes (blue; Hahn and Thomas, 2008; Kasumyan, 2008; Langård et al., 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 ($p\text{-value}<0.05$; 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

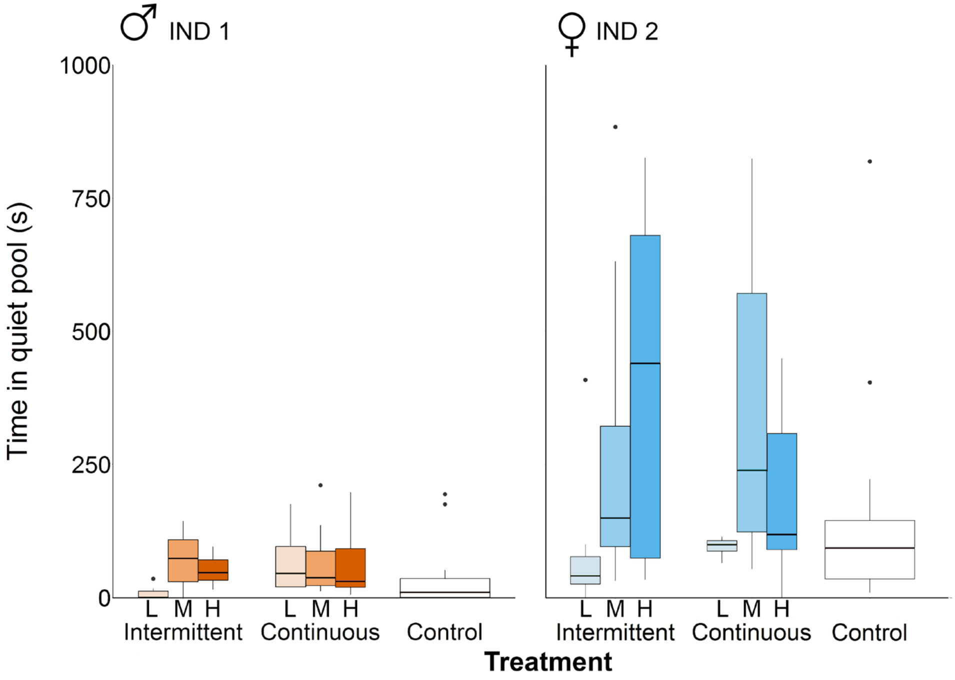


Figure 4: Porpoises spatially avoided noisy conditions. Two captive porpoises – a male (left) and a female (right) – spent time in a quiet pool when exposed to intermittent and continuous sound at three SPLs: 100 (low; L), 112 (middle; M) and 118 dB re 1 μPa (high; H); and ambient sound (Control; 79 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.

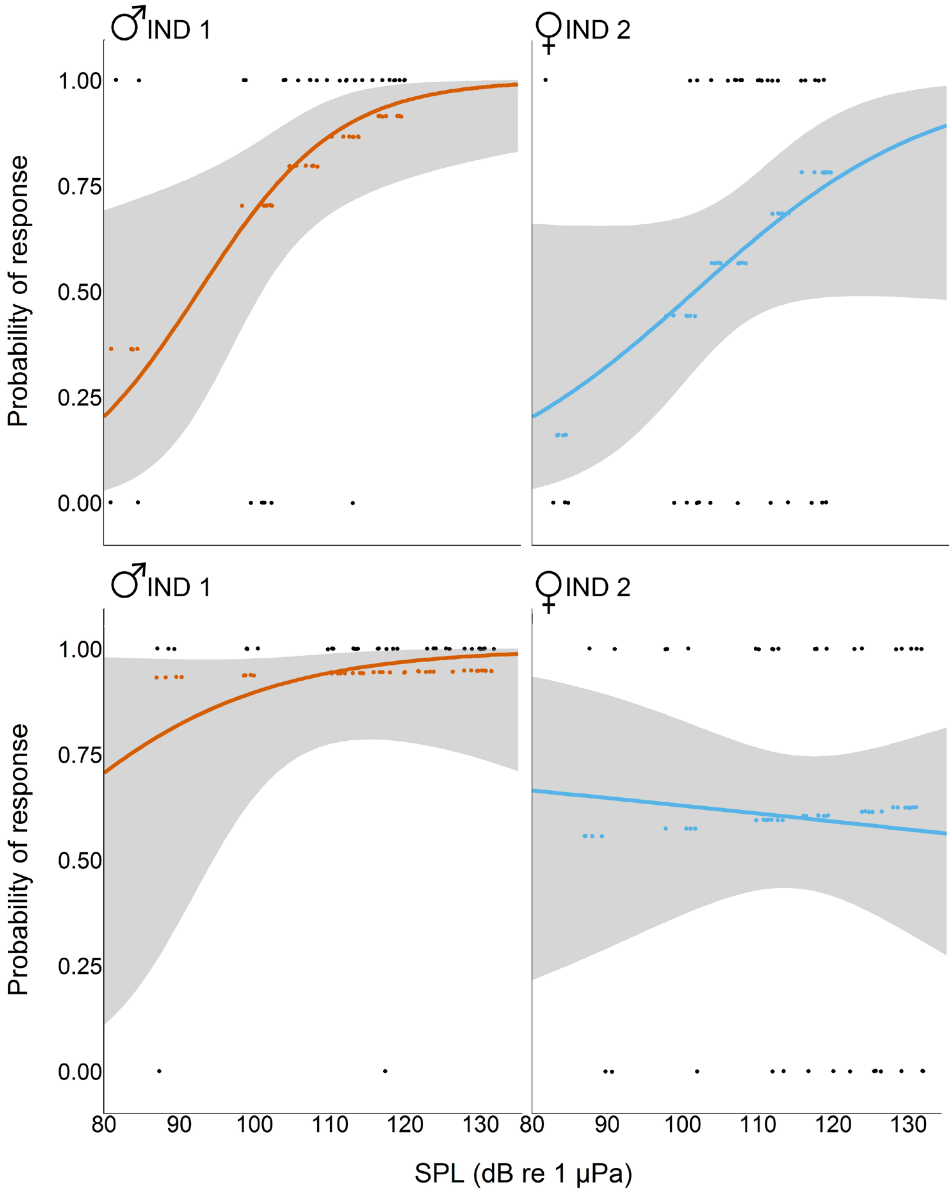


Figure 5: Acoustic dose-spatial response in two captive harbour porpoises, a male (orange, left) and a female (blue, right), to playback of intermittent (top) and continuous (bottom) brown noise of increasing SPL, measured as probability of response (more time in the quiet pool than median of control) 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.

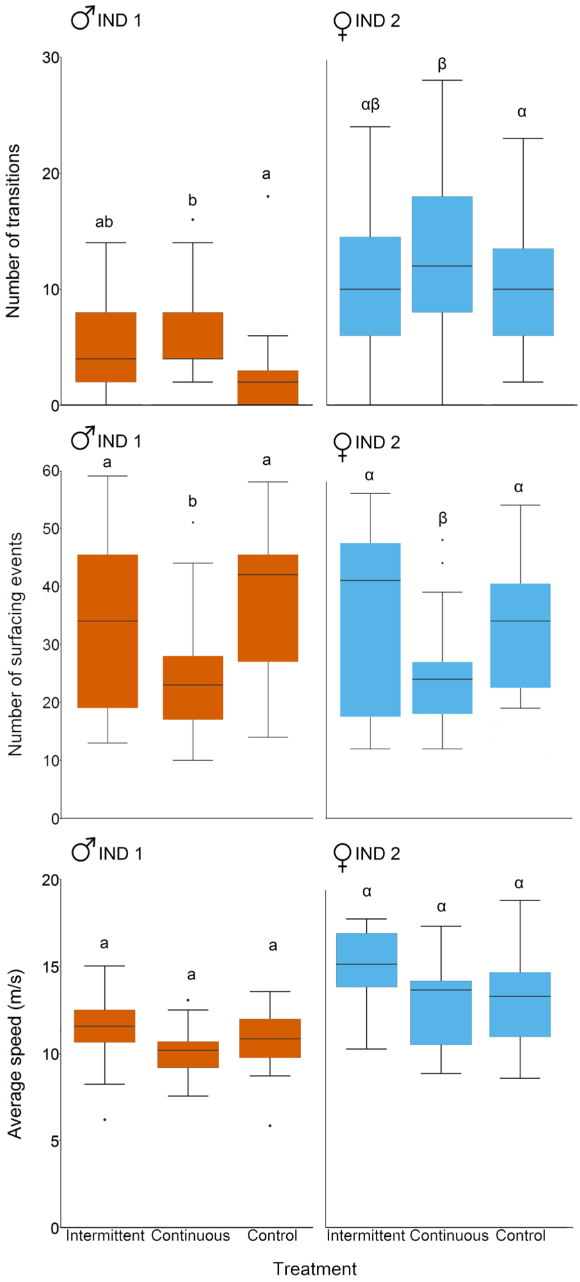


Figure 6: Exploration of behaviour under noisy conditions. Responses of two captive porpoises, a male (orange/dark grey, left) and a female (blue/light grey, right), to three exposure conditions (intermittent, continuous and control) measured as number of transitions between pools during a 15 min-exposure 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.

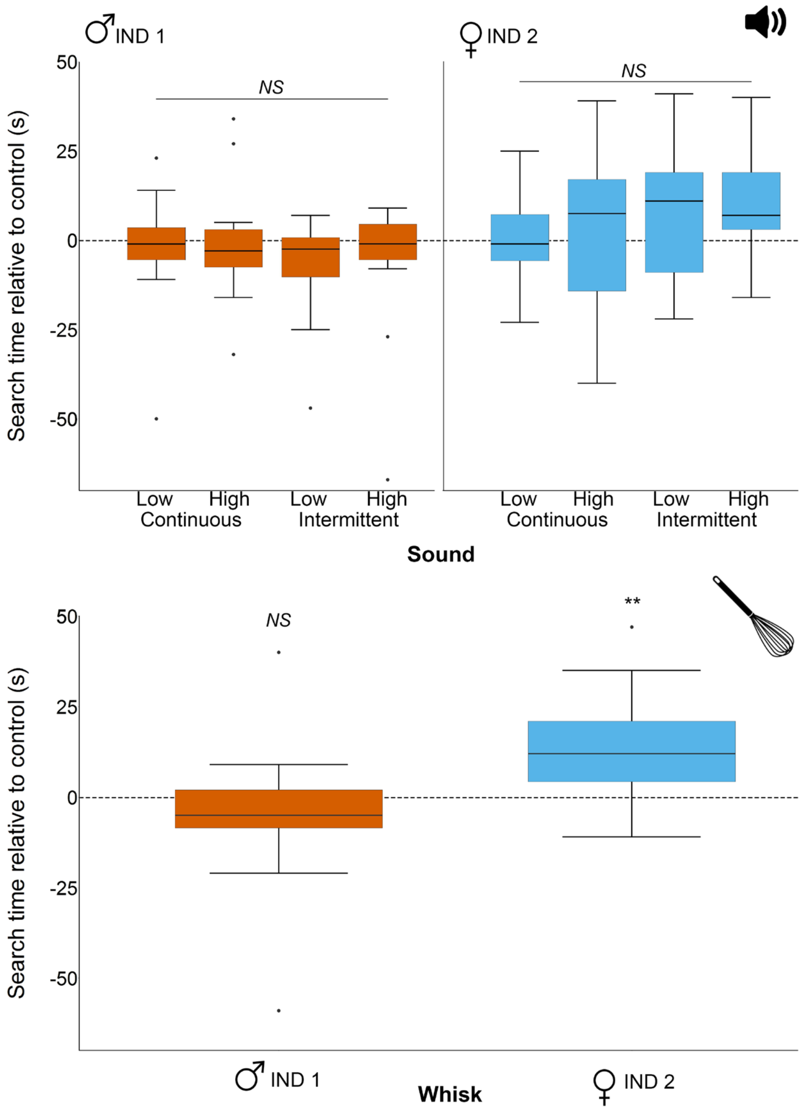


Figure 7: There was no effect of sound or whisk on search time, except for the female to the whisk. Relative search time (s) of two captive porpoises, a male (orange) and a female (blue), for a cage containing a fish in a food search task comprising three cages. Relative search time was calculated by 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: $\chi2 = 3.56$, 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

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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