AVERSIVENESS OF SOUND IN MARINE MAMMALS : PSYCHO-PHYSIOLOGICAL BASIS, BEHAVIOURAL CORRELATES AND POTENTIAL APPLICATIONS

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Aversiveness of Sound in Marine Mammals:

Psycho-Physiological Basis, Behavioural Correlates and Potential Applications

by Thomas Götz

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy
School of Biology, University of St Andrews

19th June 2008

I, Thomas Götz, hereby certify that this thesis, which is approximately 75000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Contents

Abstract

Understanding what psycho-physiological and behavioural factors influence aversiveness of sound in marine mammals is important for conservation and practical applications. The aim of this study was to determine predictors for impact of anthropogenic noise and to develop a target-specific predator deterrence system for use on fish farms. Three classes of stimuli were tested: 1.) grey seal underwater communication calls expected to be used in territorial defence, 2.) high duty-cycle moderately loud artificial sounds (some of which were based on models of unpleasantness for humans), 3.) brief, intense pulses designed to elicit the acoustic startle reflex.

Communication calls had no deterrence effect but instead caused attraction responses. Tests with high duty-cycle artificial sounds showed that food-motivated animals habituate quickly, although sound exposure caused subtle changes in diving patterns over a longer time. Field trials using the same stimuli were used to determine avoidance thresholds but also indicated that sound features like ‘roughness’ play a role. The startle eliciting stimuli, however, had the most dramatic effects. To this stimulus most seals exhibited rapid flight responses, hauled out, sensitised and showed signs of fear conditioning. Startle thresholds were found to be 80-85 dB above the assumed hearing threshold. The data showed that startle thresholds are a crucial predictor for the occurrence of strong avoidance behaviour and suggests that the startle response evolved to increase an animal’s propensity for flight. Finally, a prototype predator deterrence system based on the startle sounds was developed to repel seals whilst not affecting toothed whales. In fish farm trials, seals were deterred at close ranges but local abundance of cetaceans did not change showing that it is possible to cause differential responses between species based on differences in their audiograms.

The results are used to develop noise exposure criteria and to elucidate acoustic parameters that can be used to predict responses to anthropogenic noise.

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Chapter 1

General introduction

Marine mammals posses sophisticated underwater hearing with high auditory sensitivity over a wide frequency range (Au et al., 2000; Southall et al., 2005). While the basic functioning of the inner ear (cochlea) can be expected to be similar to terrestrial mammals, particularly the auditory systems of echolocating toothed whales (*odontocetes*) have evolved certain features that even exceed the capabilities of humans (see Nachtigall et al., 2000; Supin et al., 2001). For example, dolphins have a much higher temporal resolution and finer frequency tuning than most terrestrial mammals including humans. Since visual energy deteriorates rapidly underwater, sound constitutes the only means by which information can be transmitted over long distances. It is therefore not surprising that cetaceans and pinnipeds use sound for a variety of different purposes including communication, passive acoustic prey detection and in case of *odontocetes* also orientation and prey detection by means of biosonar (see Schusterman et al., 2000; Au et al., 2000). In the light of the high auditory sensitivity and importance of sound for marine mammal communication there has been concern about the potential impact of anthropogenic ocean noise on these animals (Richardson et al., 1995, Anonymous, 2003). Pulsed or continuous anthropogenic noise originates from many sources including seismic surveys, commercial or privately used sonar systems, drilling (e.g. for oil), wind farm operation, shipping and military activities. Direct impact of noise on the auditory system of marine mammals (e.g. temporary threshold shifts) has been investigated by several authors and attempts have been made to integrate available information to define safe noise exposure (Southall et al., 2008). An increasing amount of literature has also been published on behavioural responses to noise. Here, results seem to be more difficult to generalise and in some studies responses were found to be highly variable (see reviews by Nowacek et al., 2007; Weilgart, 2007). It seems that there is a lack of knowledge on how different factors influence behavioural responses to sound and only a few studies have ever attempted to carefully elucidate different psycho-physiological and behavioural parameters (e.g. Kastelein et al., 2006; Kastelein et al., 2005). Apart from anthropogenic noise pollution, sound sources designed to deliberately influence marine mammal behaviour have also been introduced into the oceans. These include devices operating at high-frequencies (10-40kHz) with a high acoustic output (e.g. 194 dB re 1µPa) in order to keep seals and sea lions away from fish farms or fisheries (Jefferson & Curry, 1996). Acoustic predator deterrence has been attempted with varying success but the general pattern seems to be that food motivated pinnipeds habituate quickly. However, some devices have been shown to have dramatic and long term effects (e.g. habitat exclusion) on non-target species (mostly *odontocetes*) which is now considered a serious conservation concern (e.g. Morton & Symonds, 2002).

The behavioural responses to sound can in theory be influenced by many different parameters, ranging from psychophysical factors and physiological reflexes to the biological meaning of a sound. In addition, food motivation and behavioural context at the onset of sound exposure are likely to modify responses. Given that a behavioural response can be regarded as the net outcome of changes in the physiological state of the animal, factors relating to the psycho-physiology of sound perception or physiological reflexes might be good predictors for responses to artificial sounds. An obvious example where psycho-physiology is likely to play an important role is perceived loudness. One might intuitively expect that an animal shows a stronger response when it is exposed to a higher sound pressure. Although this might be generally true it does not always need to be the case. As psychophysical data on humans have shown, perceived loudness is also influenced by acoustic characteristics of a sound other than stimulus amplitude (Fletcher & Munson, 1933). More importantly, differences in auditory sensitivity between species and taxa would have to be taken into account making a simple sound pressure level value unlikely to be a very useful predictor.

Similar to psychophysics, physiological reflexes that influence motor patterns might be good candidates for a predictor of marine mammal responses to sound. One of the best understood mammalian reflex arcs that directly influences motor behaviour is the startle response (Landis & Hunt, 1939). The basic pattern of the reflex is simple in the sense that any stimulus that reaches a certain amplitude above an animal's hearing threshold (startle threshold) within a certain amount of time (minimum rise-time) will elicit a contraction of flexor muscles (Fleshler, 1965), the strength of which is generally referred to as the startle magnitude or amplitude. In spite of this "all or nothing" nature of the reflex, the amplitude of the motor response is subject to complex modification by external and internal factors which are currently investigated on a neuronal level (Koch, 1999). Interestingly, although the startle reflex is a model system in neurobiology (Plappert & Pilz, 2001) and a diagnostic tool in medicine

(Howard & Ford, 1992) almost nothing is known about potential follow-up responses e.g. flight behaviour in animals. Also, the evolutionary origin of the reflex is still debated (see Yeomans et al., 2002 for some ideas).

Behavioural responses to sounds that have a biological meaning (e.g. communication or predator calls) might follow different principles. It is obviously crucial to understand the function of a certain call and the context in which it is produced when attempting to predict responses. This has, for example, been shown in humpback whales where animals exhibited fundamentally different movement responses to social calls versus songs (Tyack, 1983). Additionally, some artificial calls might resemble features of biological sounds and therefore elicit responses that are different from what would be expected from a meaningless sound.

The main objectives of this thesis were motivated by conservation policy: to find stimuli that could be used in an acoustic deterrence system for fish farms with a high efficiency against seals but little impact on *odontocetes* and other marine wildlife. A secondary objective was to determine factors that can be used as predictors for marine mammal responses to anthropogenic noise. Experiments were also designed in a way to provide information on more basic questions related to psycho-physiology, behavioural correlates of sound perception and function of grey seal underwater communication calls. First, the literature related to acoustic deterrence was reviewed in conjunction with available information on psycho-physiology in terrestrial and aquatic mammals (chapter 2). Then, four main topics were addressed experimentally. Chapter 3 provides an investigation into the behavioural effects of high-duty cycle (continuous) artificial sound on harbour and grey seals. This included recordings of current acoustic deterrent devices used on fish farms, some artificial control sounds and sounds based on a human model of unpleasantness. The last class of sounds was designed to increase aversiveness by means other then sound pressure level. This was also considered to be relevant for basic research questions related to the perceptual basis (e.g. critical bands) of complex phenomena like acoustic pleasantness (see McDermott & Hauser, 2004 for an earlier attempt). Chapter 4 aimed to investigate whether territorial or aggressive grey seal calls had an aversive effect on conspecifics. Chapter 5 provided the first systematic investigation into behavioural follow-up responses related to the startle reflex for any mammalian species. This also involved the first measurements of the startle threshold in an aquatic tetrapod. Finally, chapter 5 aimed to test the efficiency of the most suitable acoustic deterrence stimulus on a fish farm. It was also a test of the predictions derived from psychophysical data in chapter 2 that differential responses to the same sound stimulus can be found in pinnipeds and cetaceans because of the difference in their audiograms.

A three step approach was used to address these issues. The first step involved experiments on captive animals under controlled conditions. In the second step experiments with wild seals around haulout site were carried out and in the final phase cetaceans and seals were tested in an open habitat around a fish farm. In order to facilitate comparisons across the captive experiments and the fish farm tests a feeding station was used in captivity to instigate food motivation. This also had the advantage that a stereotypical baseline behaviour related to the feeding station was established against which behavioural alterations could be tested. Captive animals were all wild captured and the vast majority must have had experience with underwater sound in the wild, a factor which can be considered an advantage when extrapolating responses to animals in the wild. Field trials were carried out around haulout sites where conditions were relatively controlled and sound field measurements could be carried to quantify received levels. This data were also used to define potential alternative noise exposure criteria to prevent behavioural disturbance by anthropogenic noise.

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Chapter 2

Acoustic deterrent devices to prevent pinniped depredation: Efficiency, conservation concerns and possible solutions

Introduction

Worldwide farming of marine and diadromous finfish species has experienced tremendous growth rates showing a tenth-fold increase over the last three decades (FAO 2005). This huge increase in potential food resources presented in a marine environment was likely to induce interactions with predatory species. Foraging models predict that one common group of predators, marine mammals, will exploit food resources depending on their profitability and potential costs (including dive depths as major factor) an animal has to face when foraging on it (Thompson & Fedak 2001). Without any predator control methods, the costs of attacking a fish-farm are low while the profitability is high. It is therefore not surprising that these interactions occur. Nevertheless, losses have been generally regarded to be caused by only a few “rogue” individuals (SSGA 1991). Reports from British Columbia showed that a few male California sea lions *Zalophus californianus* reduced the annual steelhead trout *Oncorhynchus mykiss* run through the Ballard locks from 2500 to about 200 in less than one decade. Salmon growers reported that harbour seal predation temporarily stopped after specific individuals expected to cause the problem were removed (Morris 1996).

Seal predatory behaviour around fish farms can cause a variety of economic as well as market-related risks for the owner (Johnson 1967; Nash, Iwamoto & Mahnken 2000) which has led to the development of several anti-predator control methods. Generally speaking, these methods fall in one of the following categories: Net modifications e.g. tensioning nets to avoid seals from biting through them or adding a second net (predator net), acoustic devices to scare seals, lethal or non-lethal removals, population control and aversive conditioning (Hawkins 1985; Würsig & Gailey 2002; Quick, Middlemas & Armstrong 2004). None of these methods is free of problems: Some types of predator nets can cause tangling of predators and nonpredatory species. Lethal removals as well as population control could be dangerous for ecological reasons (particularly if the number of killed animals is underestimated), may have an impact on populations (Ross 1988), are considered ethically questionable and seem to be ineffective in some areas (Pemberton & Shaughnessy 1993). Furthermore, newly arriving individuals can quickly replace the killed animals (Ross 1988). Culling of higher order predators can also have negative impacts on predation rates by other predators. Pinnipeds forage on predatory fish species around the net pen which, in turn, potentially feed on aquaculturally important food (Fraker & Mate 1999). Finally, food aversion conditioning requires that individuals learn to associate the treated fish with sickness which may be hard to achieve when predator numbers are high (Würsig & Gailey 2002).

Acoustic deterrent devices have often been considered a benign way of dealing with the predation problem. However, its main problems appear to be habituation and the effects it has on other marine wildlife (Jefferson & Curry 1996). Recent studies on ecological problems associated with acoustic deterrents and the marine mammal auditory system enable me to investigate these problems in more detail. This review will summarize the current methodology in acoustic seal deterring, investigate the problematic biological effects and their potential ecological consequences and evaluate the potential of acoustics to control predation and movement of marine animals.

Current methods of acoustic deterrence

A variety of acoustic devices were designed over the last two decades in order to reduce or stop predation of pinnipeds on finfish farms (Table 1). In a report to the US Department of Commerce, Reeves *et al.* (1996) label low power devices operating at source levels below 185 dB re 1µPa @1m as acoustic deterrent devices (ADDs) and devices operating at source levels above that source level as acoustic harassment devices.(AHDs) This suggests (even if not intended by the authors) that devices with higher source levels "harass" seals while low-power devices just deter them (Gordon & Northridge 2002). Although there may be some psychophysical correlate to this distinction, there is no data to support the idea of "harassment" and I therefore follow Gordon & Northridge (2002) labelling all devices as ADDs "since their primary aim is to deter". In the following section and chapters different acoustic units and parameters are used depending on which question is to be answered. A brief description of the most important unit is given in appendix 4 (Glossary).

One of the first attempts to scare seals by the use of sound has been carried out in the late 1970s with captive harbour seals *Phoca vitulina* and wild grey seals *Halichoerus grypus* around netted salmon (Anderson & Hawkins 1978). The results were not promising: pure tones of unspecified source level yielded no influence at all, killer whale calls and other recorded sounds (e.g. noise) seemed to be effective for a few successive trials, but the seal response decreased quickly indicating that habituation had taken place. Anderson & Hawkins (1978) concluded that this approach has no potential for deterring seals from predating on salmon. Since early attempts yielded little, experiments with more powerful devices operating at higher source levels were carried out (published in an Oregon Sea Grant Report, Mate & Harvey 1987): Mate *et al.* (1987) used frequency-modulated pulses (8-20 kHz) of variable length at peak-to-peak (p-p) source level (sound pressure level at 1m distance) of about 187 dB re 1µPa @1m to deter harbour seals from salmon hatcheries. They observed seals to turn away when the sound was switched on. Seals sometimes even leaped out of the water and then retreated underwater quickly. In the three following years the predation rate was substantially lower and only one single seal accounted for most of the sightings in the vicinity of the device although other males were observed to pass by. The paradigm applied was to deter seals by broadcasting sounds within the most sensitive frequency range of a seal. However, although the device seemed to prevent huge recruitment of new animals, in the fourth year the predation rate returned to its original level. The device now even seemed to attract these seals yielding the opposite effect as originally intended. Hearing loss might be one explanation, because the sound of an ADD might be of sufficient intensity to damage the auditory system of seals (Reeves *et al.* 1996), but it is likely that habituation or even a conditioned response resulting from the association of the sound with a profitable food source was responsible for this result. Such a “dinner bell” effect was reported by other studies as well, e.g. Geiger & Jeffries (1987) observed that the originally aversive sound has become a conditioned reinforcer. The time until habituation occurs varied between different studies and study sites. Harvey & Mate (1987) tried to establish an acoustical barrier (using a similar device) to deter seals from feeding on migrating salmon and found it to be effective for only a few days, while Rivinus (1987) reported that only in the third year one or two individuals returned.

Further work on sound as a deterrent has been done on the *Otariidae* (eared seals). Akamatsu *et al.* (1996) investigated behaviour of captive Steller seal lions while freely swimming in the pool and while feeding on salmon attached to a net. They tested an iron drum (0.5-2 kHz, 210 dB re 1µPa) and different playback sounds at maximum source level (rms) of 165 dB re 1µPa. Among playback sounds killer whale calls yielded little effect, frequency–modulated sweeps (1-4 kHz, 1 s duration, 1 s inter-stimulus interval) repelled juveniles and pure tones (8 kHz, 5 s duration, 5 s inter-stimulus interval) were successful in repelling all animals. Only the iron-drum was able to deter males in the feeding trial which might be due to the high source levels of its sounds.

While at least some work has been done on negative effects of commercially manufactured ADDs on non-target-species, our knowledge of efficiency on target-species (pinnipeds) is patchy. Table 1 gives a summary of the acoustic characteristics of ADDs that were sold commercially over the last years. Yurk & Trites (2000) tested ADDs produced by the companies Airmar and Ferranti-Thompson in an attempt to keep harbour seals from feeding on out-migrating salmon under a bridge. The Airmar dB Plus II device indeed yielded a decrease of predation rate in 7 successive trials, but further trials were not carried out. The Ferranti-Thompson device was only tested once yielding a decrease in the number of seals in comparison to the control trial on the following day but seal numbers were still high in comparison to earlier control trials. Jacobs & Terhune (2002) tested an Airmar dB Plus ADD (consisting of an array of four transducers) by chasing harbour seals from a haul-out in the water with an approaching boat and consecutively monitoring surface positions of seals swimming in the water (measured peak to peak source level was only 172 re 1µPa @ 1m). They found no differences between control and sound exposure sessions. In another experiment the authors could not find any effect of an acoustic barrier consisting of Airmar ADDs on harbour seals approaching a haul-out site. Similarly, acoustic deterrent devices used to protect salmon runs (National Marine Fisheries Service 1995) and fish farms (Norberg 1998) had little effect on *otariids* although in some cases recruitment of new individuals was successfully prevented (National Marine Fisheries Service 1995). One of the manufacturers (Ferranti-Thompson) stopped production of their ADD, but Ace-Aquatec took over the production of a seal scarer that is based on the same transducer and developed a triggering system based on sensing salmon panic movements in the pen when a seal is present (Ace-Hopkins 2002). This approach reduced the duty-cycle substantially. In an internal company report Ace-Hopkins

Manufacturer	**Ferranti-Thomson**	**Ace-Aquatec**	**Airmar Technology Corporation**	**Terecos Ldt**	**Lofitech (older models by SIMRAD)**
Model	Ferranti-Thomson MK2, Mk3 & 4X Seal scrammer	Ace-Aquatec “Silent Scrammer”	Airmar dB Plus II	Terecos type DSMS-4	Lofitech “universal scarer” or “seal scarer”
Source level (re 1 µ Pa)	195dB @ 27 kHz[1] (peak) for MK2 model 200dB @ 25 kHz (n/a) for 4X model	193 dB @ 10kHz (rms)	192 dB @ 10.3KHz (rms)	178 dB @ 4.9 kHz[1] (rms)	191 dB @15 kHz (n/a)
Frequency structure	pulses centred at 5 different frequencies arranged in 5 pre-set sequences (pattern of jumping frequencies) which are chosen randomly[2]	pulses centred at 28 different frequencies (pattern of jumping frequencies) arranged in 64 sequences which are randomly chosen	more or less sinusoidal: 10.3 kHz (2nd harmonic 43 dB weaker)	complex (randomized sequences of different components): tonal blocks (with harmonics) forming up and down sweeps (fundamental from 1.8 kHz-3 kHz), randomised sequences of continuous and time variant multi-component blocks (2.4 kHz-6kHz), continuous tonal blocks forming up and down sweeps combined with continuous multi-component blocks [1]	15 kHz (tonal, narrow-band)
Temporal pattern	20 ms pulses repeated every 40 ms in trains of 20s duration[2]	3.3-14 ms long segments in 20 s long trains	1.4 ms long segments at 20 ms intervals in 2.25m long trains; 4 transducers produce these trains in an alternating pattern	depending on operation mode: 8ms segments in sequences of eight or 16ms segments in sequences of 5; variation possible due to randomisation software[1]; trains from 200ms to 8 s long[2]	500ms pulses in 6s trains long trains
Duty cycle	3 %[2] max.5.5 scrams per hour	activity-dependant (50% if trigger is released, but max 18 times per hour)	40-50 %	ca. 50 % [2]	20-25 %
Energy in the ultrasonic range	Yes, at least up to 40 kHz[1]	more than 165 dB at 30 kHz;145 dB at 70 kHz	145 dB up to 103 kHz	less than 143 dB above 27 kHz [1]	occasionally one harmonic depending on battery status
Commercially available	no	yes	through Bennex	Yes, also rental scheme	yes
Reference	Yurk & Trites (2000)[1] manufacturer‘s description cited in Gordon & Northridge (2002)[2]	Lepper *et al.* (2004)	Lepper *et al.* (2004)	Lepper *et al.* (2004)[1] Reeves, Read & Nortabartolo di Sciara.(2001) [2]	Reeves, Read & Nortabartolo di Sciara (2001)

Table 1: Acoustic characteristics of currently used acoustic deterrent devices (ADDs). If multiple source were used numbers in superscript indicate the citation the information was taken from. All dB values are based on a reference value of 1uPa.

(2002a) presents a number of trials with his device at different salmon farms in Scotland (potential predators are grey and harbour seals). At the first farm site the device was initially set to the so-called listening mode (which means that it only detects attack events, but does not emit any sound) for about 7 weeks with 37 seal-damaged/killed fish occurring during that time. After switching his device to scram mode (meaning that it emits sound when detecting quick movements of salmon in a pen) for the following seven weeks only 7 killed fish could be found. At a second salmon farm his device yielded no success and at a third and fourth he reported that predation dropped to zero, but unfortunately he did not present any detailed data on control trials or study design and did not carry out any statistical analysis. Indirect information on the effectiveness of current ADDs can be obtained by analysing experience discussed in workshop reports and questionnaire surveys. Reviews summarizing information on long-term efficiency of these devices report varying success: While Fraker (1996) reported short-term effectiveness (6-8 weeks) measured as a reduction of fish mortality, Reeves *et al.* (1996) report ADDs to be efficient for up to 2 years. Quick et al. (2004) carried out a questionnaire survey on Scottish fish farms but did not analyse the perceived efficiency of different ADDs by model due to differences in their out deployment. They identified 7 brand names probably covering the whole spectrum of commercially available devices. Only 23% of the fish farmers reported them to be very effective, 50% reported moderate, 15 % poor and 7% little efficiency. Just like in other areas (Mate *et al.* 1987) some of the farmers believed them to even attract seals. Reeves *et al.* (1996) and Iwama *et al.* (1998) reviewing available information from scientists and stakeholders concluded that current seal scarers seem not to be effective enough to recommend their future use, especially with respect to possible impacts on non-target species. However, some fish farms in Scotland reported ongoing efficiency of their seal scarers (Ross 1988). The reasons for such differences might be diverse, ranging from the animal's foraging motivation to differences between populations in behaviour to human actions or differences in sound propagation characteristics of the habitat.

Ecological impacts on target and non-target species

Acoustical devices could potentially cause negative impact on animals in three different ways (Gordon & Northridge 2002):

- Damage to the ear due to high sound pressure
- Masking effects which might affect communication, orientation or prey detection

- Behavioural reactions e.g. avoidance behaviour which could lead to an exclusion of animals from parts of their habitat.

Species of concern

Any animal that can perceive ADD sounds can potentially be affected by them. A high-power ADD (e.g. Ferranti-Thompson 4x) can be audible to a harbour porpoise (*Phocoena phocoena*) for up to 10 km under low ambient noise (Taylor et al. 1997). A harbour seal could potentially hear a device with a source level of 175 dB re 1µPa @ 1m at distances of 1.4 km to 2.9 km in quiet conditions (Terhune et al. 2002). In some species we have little knowledge of how they would react to ADD sounds. An exception are *odontocetes* where avoidance reactions have been described in detail (see below). Cetaceans rely on sound for communication and toothed whales (*odontocetes*) use active sonar (echolocation) for prey detection and orientation (Au 1993). Many fish species use sound for communication (Zelick et al.1999) and some *clupeids* have very broad hearing ranges (Mann et al. 1997). Thus, it is possible that some fish species are also affected.

At first glance impacts on target-species may seem to be of little concern since ADD's are used to deter seals. However, if ADDs caused serious hearing damage this would be a problem not only from a conservation point of view, but also from a site-manager's perspective since efficiency of the devices would be dramatically reduced. It is also important to note that hearing damage first affects the outer hair cells (OHC) in the cochlea which only leads to a moderate, almost not measurable rise of the hearing threshold (deafness). However, even at this level of hearing damage the cochlea amplifier is damaged causing a diminution of the dynamic range and a loss of the frequency discrimination ability (see Moore 1997 for a review on psychophysical effects of hearing damage). Since *pinnipeds* might rely partly on passive acoustics for prey detection (Schusterman *et al.* 2000) psychophysical effects caused by the loss of the cochlea amplifier could be as hazardous as the actual threshold shift since a reduced ability to classify sounds could make these animals even more dependent on predictable food sources like farmed fish.

Hearing damage

There is some controversy over the effects of ADDs on cetacean and pinniped hearing. While manufacturers (Ace-Hopkins 2002b) reject the possibility of hearing damage caused by their ADDs, researchers come to a different conclusion and often state that this concern can currently neither be proven nor dismissed (Gordon & Northridge 2002). Hearing damage occurs first as a temporary but fully recoverable shift of the hearing threshold (temporary threshold shift=TTS). The hearing threshold is the sound pressure level that is just audible to the animal. As a result of exposure to higher intensity or longer duration acoustic stimuli, recovery may not be possible and the threshold shift becomes permanent causing chronic damage (permanent threshold shift=PTS). The risk of hearing damage is considered to be a function of sound pressure level and exposure time (Eldred et al. 1955). For instance a sound with a short duration can be safely presented at a higher source level than a longer one. It has been suggested that stimuli of equal acoustic energy carry the risk of causing similar damage (Eldred et al. 1955). Therefore, sound exposure level (SEL) or energy flux density was suggested as a measure for defining safe exposure levels: SEL= SPL +10 $\log_{10}$ (exposure time in seconds). However, note that data on terrestrial mammals suggests that equal energy criteria underestimate the risk at least for sound pressures close to a certain critical level of about 135 dB re 20µPa (Danielson *et al.* 1991). Since no direct measurements of permanent threshold shift are available for marine mammals, human data or extrapolation from TTS (temporary threshold shift) has to be used to draw any conclusions. Southall et al. (2008) reviewed available literature on TTS in marine mammals and developed a set of noise exposure criteria trying to define safe sound exposure levels for different taxa. These values appear to be relatively high which might be the result of several factors. Many studies on TTS in *odontocetes* were carried out with animals at the Space and Naval Warfare Systems Centre (SPAWAR) centre in the US (Schlundt, et al. 2000, Finneran et al. 2005). The subjects tested by Finneran et al. (2005) did have slightly higher hearing thresholds at the test frequencies compared to healthy animals: while the threshold in one of Finneran et al.’s animals (“Ben”) at 3kHz was about 90 dB re 1µPa (read from fig 3) Johnson (1967) reported a threshold of 76 dB re 1µPa. The same animal showed clear signs of permanent hearing damage in the frequency band between 10 and 40 kHz (note the clear notch in the audiogram of “Ben” in fig 3 in Finneran et al. 2005). It is however true that this was not at the test frequency. More importantly, one needs to consider that other *odontocete* species have much lower hearing thresholds at 3 kHz (e.g. killer whales; see fig 1) and might therefore develop TTS at lower sound exposure levels. Also, bottlenose dolphin hearing thresholds can be as low as 43 dB re 1 µPa in the ultrasonic range (Johnson, 1967); so it might well be that a healthy bottlenose dolphin (unlike the subject "Ben") that is exposed to signals at higher frequencies develops TTS at much lower exposure levels. I believe that values for the onset of TTS from these experiments might therefore be too high if they are not referenced to the hearing sensitivity of the subject under test conditions. In humans, low-frequency noise (300-600Hz) causes lower level of TTS compared to noise that falls in a frequency band (2400-4800 Hz) where hearing is more sensitive (Ward et al. 1959). Marine mammal researchers tend to call for caution when extrapolating information from human data (e.g. Southall et al. 2008). However, while marine mammals have adaptations to aquatic hearing there is currently little evidence that the basic functioning of the cochlea is fundamentally different from terrestrial mammals. For instance, a closer look at the TTS data reveals that *odontocetes* do not seem to have a much larger dynamic range than humans. Finneran et al (2005) proposed an SEL of 195 dB re 1 µPa^2-s as the threshold for onset of TTS for mid-frequency tones. The measured hearing thresholds of the two experimental subjects at the exposure frequency were between 80 and 90 dB re 1µPa. The difference between the onset of TTS and the hearing threshold for the tested animals is therefore approximately 110 dB which is in the same order of magnitude as in humans. While Southall et al. (2008) proposed a weighting procedure for different species groups, they did not compensate for differences in hearing abilities of the subjects under the specific test conditions in each of the reviewed studies. This would however be important in cases where subjects were not always tested at the frequencies where their hearing was most sensitive. An alternative approach leading to lower criteria could be based on SEL calculations expressed in sensation levels above the hearing threshold. I argue that this is physically correct because sound exposure level (SEL) calculated as SEL=SPL+10log (exposure time in s) (see Madsen 2005 for equation) includes a sound pressure term (SPL) that is based on the decibel scale. Since the dB is a relative unit, using the hearing threshold as a reference value would make sense. In that case the hearing threshold should be subtracted from the SEL value for the onset of TTS leading to a sound exposure level expressed in units of sensation levels. This concept has also been used by Kastak et al. (2005) for comparative purposes. Although Kastak et al. (2005) caution that they did not intend "to make any inferences about the relationship between an auditory threshold and the sound energy resulting in TTS", the differences in onset of TTS found between different species could be well explained by differences in their hearing thresholds. In humans contours of perceived equal loudness run parallel to the hearing threshold over a large frequency range and are only compressed at the edges of the hearing range (Fletcher & Munson, 1933). Therefore if onset of TTS follows these contours then the suggested SEL expressed in terms of sensation levels may overestimate potential impact zones. Given the general controversy, it may however be fruitful to consider multiple scenarios when predicting TTS or PTS. To illustrate this further I will now give potential damage zones for seal scarers based on Southall et al.'s (2008) criteria but also provide estimates based on a sensation level type of approach and extrapolation from human data. In the following section the term SEL refers to the normal sound exposure level while "SEL-sensation level" refers to sound exposure levels based in the hearing threshold as a reference value.

Temporary threshold shifts (TTS)

Finneran et al. (2005) showed that sound exposure levels (SELs) of around 195 dB re 1 µPa^2-s caused TTS in two bottlenose dolphins at frequencies of 3 kHz. The sound exposure level of a Ferranti-Thomson Mk 2 or Ace-Aquatec seal scarer emitting a 10s burst (= the energy of a 20s scram produced by a Ferranti-Thomson Mk 2) at a source level 193 dB re 1 µPa @ 1m would be 203 dB re 1 µPa^2-s. The difference between the sound exposure level causing onset of TTS and sound exposure level of the ADD would therefore be only about 10 dB. Assuming spherical spreading TTS would only be caused at 2-3m distance from the device. However, data on harbour porpoise suggested that SELs as low as 164 dB re1µPa^2-s can cause TTS (Lucke et al. 2007). The signals tested by Lucke et al. (2007) were short transients with broad spectra, however, most energy was below the most sensitive hearing range of the harbour porpoise. Assuming spherical spreading and absorption losses of about 5dB per km, TTS would be caused in animals closer than 87m from the transducer. The lowest SEL causing TTS in a pinniped was found to be 183 dB re 1µPa^2-s in a harbour seal (Kastak et *al.* 2005). Temporary threshold shifts in pinnipeds would therefore occur at any distance equal or closer than 11 m to the seal scarer. It should be noted that it is not known how TTS in marine mammals develops in response to repeated exposure to several emissions by an ADD.

As I argued earlier the possibility that TTS can be caused at lower SELs at frequencies where hearing is more sensitive should be taken into account. Studies on odontocetes found SELs between 193 and 213 dB re 1 Pa^2-s to cause mild to moderate but fully recoverable TTS (Au et al. 1999; Finneran *et al.* 2000; Schlundt *et al.* 2000; Finneran *et al.* 2002; Finneran *et al.* 2005). These values were between 110-132 dB re (hearing threshold in Pa)^2–s. As mentioned earlier Finneran et al. (2005) suggested an onset-TTS criterion of 195 re 1 µPa-s. The tested subjects had hearing thresholds between 80 re 1 µPa and 90 dB re 1 µPa at the exposure frequency under quiet conditions (read from fig 3 in Finneran et al. 2005). This would mean that "SEL-sensation level" of 110-115 dB re (hearing threshold in Pa)^2-s would cause onset of TTS. Using the 115 dB re (hearing threshold in Pa)^2-s value and adding the most sensitive measured hearing threshold in the frequency band between 10-35 kHz (where most ADDs operate) for each species will then give sound exposure levels (SEL) that marks onset of TTS. The hearing thresholds I used in this calculation were 37 dB re 1 µPa for the harbour porpoise (Kastelein *et al.* 2002), 43 dB re 1 µPa for the bottlenose dolphins (Johnson 1967), and 30 dB re 1 µP for killer whales (Hall & Johnson 1971). The respective onset-TTS levels in the most sensitive hearing range would then be 145 dB re 1µPa^2-s, 152 dB re 1µPa^2-s and 158 dB re 1µPa^2-s. Continuous exposure for 10s at 194 dB re 1 µPa (SEL=204 dB re 1 µPa^2-s) would therefore result in TTS zones of approximately 438m for harbour porpoises, 180m for bottlenose dolphins and over 622m for killer whales (based on spherical spreading and 5dB per km absorption).

Permanent threshold shift (PTS)

The noise exposure criteria published by Southall et *al*. (2008) would suggest permanent injury in most *odontocetes* at SELs of 198 dB re 1µPa^2-s for multiple pulses and 215 dB re 1µPa^2-s for continuous noise. Since some seal scarers emit pulse trains (e.g. Airmar; see table 1) I used the criterion for multiple pulses. Using the assumptions mentioned in the previous section this would mean that hearing damage in *odontocetes* would be caused if an animal is closer than 2m from the sound source. However, if an animal stays close for a longer time, repeated exposure to several bursts could increase the risk. For example, if one assumes continuous exposure for about 10 min then damage zones would be 15m for *odontocetes*. Using Southall et al. (2008) criterion for pinnipeds (multiple pulses: 186 re1 µPa^2-s) would result in a damage zone of 8m (assuming spherical spreading).

Alternatively, one might try to calculate damage zones using the mentioned “SEL-sensation level” approach. This was based on the sound exposure level-sensation level of 115 dB Pa^2-s for odontocetes derived from the results on bottlenose dolphins (see previous section). The predictions of PTS from TTS are based on suggestions by Kryter (1994) stating that exposure levels that cause TTS of more than 40 dB carry the risk to result in permanent damage. Data on humans showed that threshold shifts of about 40 dB are correlated with an increase of the exposure level by approximately 20 dB (Ward et al. 1958). Based on these considerations and the data presented in the preceding paragraphs, damage zones within which (PTS) would occur would be 20m, 40m and 85 m for the bottlenose dolphin, harbour porpoise and killer whale respectively.

Lucke et al. (2008) found onset of TTS in harbour porpoises at sound exposure levels of 164 dB re 1µPa^2-s; therefore using the criteria described in the previous section PTS could be expected at levels of 184 dB re 1µPa^2-s. Assuming spherical spreading and previously mentioned absorption, exposure to one burst of an ADD (SEL=204 re1µPa^2-s) would only be sufficient to damage hearing at distances of less than 10m.

A few alternative approaches using data from humans should be considered, too. Taylor et al. (1997) applied human damage risk criteria (DRC, critical value of 130 dB above the hearing threshold) for single exposures adjusted to the hearing range of the harbour porpoise (Anderson 1970) to predict a zone of 7 m within which PTS would occur (in response to a high power Ferranti Thomson Mk 4x) . Studies on terrestrial mammals confirmed that permanent hearing damage occurs quickly when they are exposed to sound pulses at 130-140 dB above the hearing threshold (Danielson *et al.* 1991; Henderson *et al.* 1991). Using the 130 dBA criterion and more recent data on harbour porpoise hearing (Kastelein *et al.* 2002), the damage zone would be 30 m (see Gordon & Northridge 2002).

Longer-term exposure (e.g. >1.5 minutes/day) requires different calculations to be made. Gordon & Northridge (2002) used Kryter’s (1994) damage threshold of 115 dB above the hearing threshold for exposures of up to 1.5 min. They yielded PTS ranges between 79m and 562 m for a high power device (200 dB re 1µPa @ 1m) and values between 40 and 281 m for a 194 dB re 1µPa ADD depending on the species’ hearing threshold.

Long-term exposure over months or years requires even more conservative criteria. Accepted noise levels at industrial workplaces are about 85 dB above the hearing threshold (NIOSH 1998). Taylor *et al.* (1997) used an even more conservative threshold of 80 dBA. The zone where this value is exceeded would be over a kilometre for an Airmar device. In areas with dense fish farming animals could be exposed to these levels for an extensive amount of time. As studies on humans have shown initially harmless TTS can turn into PTS if recovery periods are insufficient or non-existent (Kryter 1985).

Finally, there is a chance that fish will be affected by ADD’s. For farmed salmon there seems to be little risk of impact since the species is very insensitive to frequencies higher than 1 kHz. Mate *et al.* (1987) could also not find any behavioural response or influence on egg fertility using frequencies higher than 800 Hz. Furthermore, even marine fish species with specialised high-frequency hearing (e.g. *clupeids*) have thresholds that are at least 20 dB above those of marine mammals at frequencies higher than 2 kHz (see fig 1). Damage of the hair cells has been found in cod exposed to sound pressure levels of 180 dB re 1 µPa for several hours (Enger 1981), in oscars *Astronotus ocellatus* that were exposed to 300 Hz sine wave sounds of the same source levels (Hastings *et al.* 1996) and snappers that were repeatedly exposed to airgun emissions (received levels up to 180 dB re 1 µPa, peak frequencies between 20-100 Hz) used for seismic surveys (McCauley, Fewtrell & Popper 2003). Smith et al. (2004) showed that hearing generalist and specialists among fish exhibit different susceptibility to noise exposure. While an increase in noise level caused a continuous increase in TTS in goldfish this was not the case in Tilapia (a hearing generalist). These studies used signals within the most sensitive hearing range of the species making it difficult to draw conclusions about the effect of higher frequency signals as used in ADDs. It is also important to note that in contrast to mammals hair cells in fish can re-grow after acoustic trauma and therefore any damage caused by exposure to loud sound might only be temporary (Corwin, 1981, Lombarte et al., 1993; Popper & Hoxter, 1984). However, such a temporary hearing damage may still have some kind of fitness consequence for fish. However, given that even hearing specialists among fish have low auditory sensitivity above 10 kHZ (with a few exceptions) current ADDs are probably unlikely to damage hearing of most fish species.

In conclusion, effects of current ADDs on fish without specialized hearing are less likely (although this possibility should be investigated by future research), but effects on cetaceans and seals are relatively clear. Depending on the assumptions made, the acoustic characteristics of the device and the species hearing sensitivity, distances within which ADDs can cause permanent or temporary hearing damage range from negligible to highly-relevant. So depending on which assumptions one accepts there may or may not be a need for action. Damage ranges for killer whales are larger since their hearing thresholds are lower in the frequency bands used by ADDs. Since all calculations are based on relatively few data (mostly only one or a few animals were measured) the most precautionary approach should be considered. Current acoustic deterrence methods, particularly when used extensively, may damage hearing of target species but also non-target-species on a long-term basis. This would reduce fitness of the individuals involved and, if large parts of the population were affected, hearing loss could lead to effects on a population level. Therefore, it is crucial to improve our knowledge on marine mammal hearing and possible effects of noise and implement efficient mitigation procedures.

Masking

Masking means that the detection of one sound (signal) is influenced by a second sound (masker). Fletcher (1940) found that masking effects in mammals depend on the bandwidth of the masker (centred at the frequency of the signal) until it reaches a so-called critical bandwidth (CB). Therefore, noise only masks a signal if it contains similar frequencies to the signal of interest. Critical bandwidths in marine mammals are generally below 10 % of the signal's centre-frequency (Richardson et al. 1995). Additionally, masking effects are attenuated if the masker and signal come from different directions. Terhune (1974) found that the harbour seal's minimal audible angle for clicks is 4.5°. Bottlenose dolphins can distinguish sound sources that are presented at angles of less than 3° apart (Renaud & Popper 1975). Furthermore, bottlenose dolphin hearing is directional (Au & Moore 1984) which increases the capability of detecting signals in noise if noise source and target sound are spatially separated. Therefore, cetaceans and pinnipeds may successfully avoid masking effects in some cases. There may be a potential to affect marine mammal communication networks (Janik 2005) e.g. by decreasing detection distances of communication signals. Direct measurements have to be obtained to get detailed information, especially in the frequency range at which communication or echolocation sounds overlap with sounds produced by ADDs.

Hearing abilities (e.g. localization and frequency discrimination) in the majority of fish species are less sophisticated than in mammals (Fay & Popper 1999) which might make them more prone to masking effects. Elevated detection thresholds as a result of masking have been shown in hearing generalists as well as specialists (Vasconcelos et al., 2007; Wysocki & Ladich, 2005). Also, fish species seem to differ in their susceptibility to masking (Ramcharitar & Popper, 2004). A neuro-physiological study on goldfish (a hearing specialist) showed that responses of nerve fibres to tones between 400 and 800 Hz can be suppressed by maskers of a broad range of frequencies essentially covering most of the hearing range (Fay, 1991). These studies show that masking of communication signals in fish is a definite possibility. However, most communication signals in fish are fundamentally lower than the frequency band where most ADDs operate (see Zelick et al. 1999). Therefore, current acoustic deterrent devices are probably less likely to mask communication signals in fish.

Habitat exclusion

Behavioural reactions of marine mammals to noise have been well documented for a variety of noise sources (Richardson *et al.* 1995). Behavioural avoidance responses to ADD’s leading to an exclusion from the habitat have been studied in harbour porpoises and killer whales. Olesiuk *et al.* (2002) carried out a study in the Broughton Archipelago (British Columbia) investigating effects of the Airmar ADD on harbour porpoise distribution in the respective observation area. When the ADD was switched on the number of animals detected dropped significantly to 1.9% and 3.8 % of values in control sessions, depending on the sector scanned. Porpoises were completely excluded from an area of 400m radius around the ADD and the number of sightings was still below 10 % of the expected value at ranges between 2500 and 3500m from the device. Johnston (2002) carried out an additional experiment using an accurate theodolite tracking method and found that porpoises did not approach an emitting ADD closer than 645 m (received level at this distance would be 128 dB re 1µPa). The average closest approaches were 991m (in contrast to 363 during control) and significantly less porpoises could bee seen within a range of 1500m. In addition, porpoises moved out of the area after the ADD was switched on. Morton & Symmonds (2002) reported a dramatic decrease in killer whale sightings in Johnston Strait, Canada after ADDs (most likely different brands) had been introduced on fish farms and a recovery of sighting rates after fish farmers stopped using them. This change did not correlate with changes in local food availability. Interestingly, no differences in sightings of seals were observed. This study covers a period of 15 years and therefore indicates that cetaceans, in contrast to seals, did not habituate to ADDs. Morton (2000) found that Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) abundance decreased after ADDs were introduced in the area. Robertson (2004) monitored harbour porpoises in the vicinity of fish farms in Orkney visually and acoustically by logging click trains with automatic click detectors. She found that fewer detections of porpoises occurred in the zone considered to be affected by the ADDs. More porpoises were logged in the control zone away from the farm and no obvious influence on use of the nearby haul-out site by seals could be found.

Little information is available on behavioural disturbance of fish, but these effects might be limited to species with sophisticated hearing. Kraus et al. (1997) found that catch rates in gillnets with pingers were lower, but a causal relationship to the sound could not be proven. Experiments with salmon smolts showed that a 10 Hz signal 114 dB above the hearing threshold caused an avoidance reaction while a 150 Hz signal did not (Knudsen, Enger & Sand 1994). Wardle et al. (2001) used video observations and tagging methods to monitor behaviour of cold water reef fish (including Pollack *Pollachius pollachius*) during airgun emissions. All fish showed C-starts (a reflex initiated by quick motor neurons) in response to every sound emission at peak to peak sound pressure levels higher than 195 dB re 1µPa but directional avoidance responses only occurred when fish could see the explosion visually. However, the lack of a clear behavioural response does not mean that the exposure levels were not harmful to the fish. Kastelein et al. (2007) tested behavioural responses of a variety of North Sea fish species to several commercially available pingers. The authors concluded that particularly pingers with signals higher than 10 kHz are less likely to affect fish species.

The described studies showed that several cetacean species were excluded from their habitat which highlights a serious management concern, while influence on fish is difficult to estimate. In conclusion at least the Airmar dB Plus II device seems to have stronger long-term effects on non-target species than on seals. However, ADDs with more substantial energy in the ultrasonic range (Ferranti-Thomson, Ace Aquatec) can be expected to be even more problematic.

Acoustical devices to avoid harmful interaction of cetaceans with gillnet fisheries: Lessons that can be learned from pinger studies

Although the problem of deterring marine mammals from gillnets is generally different from deterring them from feeding on fish farms, there are similarities making it interesting to compare both problems. While a pinger can be judged as efficient if it draws the animal’s attention to the presence of a net and enables it to show an obstacle avoidance reaction, an acoustic deterrent device has to be aversive enough to overcome the drive of the animal to feed on a profitable food source. Additionally, a large number of studies trying to find aversive sounds to keep harbour porpoises away from nets can give valuable insights into signal design since a seal scarer should ideally not use sounds that deter porpoises.

Pingers can reduce by-catch rates of harbour porpoises in gillnets substantially (Kraus *et al.* 1997). Field observations around a net with pingers indicated that harbour porpoises avoided an area of about 130 m around the sound source, a PICE pinger produced by Loughborough University /UK operating at source level of 145 dB re µPa at 1m (Culik *et al.* 2001). There is however some evidence for habituation of porpoise avoidance responses to pingers when responses are monitored over several weeks (Cox *et al.* 2001). A recent study on two captive porpoise tested different acoustic alarms operating a frequencies between 100 and 140 kHz (source level between 128 and 153 dB re 1µPa) and found quick habituation. However, population level effects of pingers with respect to bycatch reduction seem to be present over longer times e.g. Larsen & Krog (2007) showed that pingers were effective over a 10 year period . This could have to with the fact that pingers may still be able to reduce bycatch even though animals habituated (e.g. by drawing the animals’ attention to a potential obstacle). Alternatively, it may be possible that porpoises did simply not habituate or that the level of the avoidance response decreased but some effect was still present in the vicinity of the device. Playback experiments with porpoises showed that multi-harmonic sweeps with most energy between 55 and 70 kHz (Kastelein *et al.* 2000) as well as ultrasonic broadband pulses (60-80 kHz) and pure tones (70 kHz) caused strong avoidance reactions (Kastelein 1997). Another study on captive porpoises showed that harmonics above 10 kHz were crucial to cause aversive responses (Kastelein 1995). Harbour porpoise behaviour can differ from that of bottlenose dolphins which have been observed to occasionally approach loud sound sources (Goodson 1997) and do not appear to avoid gill-nets with pingers in a very strong way (Cox *et al.* 2004). In the study by Cox et al. (2004) the bottlenose dolphins were assumed to be naïve to pingers, therefore, habituation cannot account for this behaviour. Kastelein et al. (2006 a) found that a captive striped dolphin *Stenella coeruleoalba* did not respond to an acoustic alarm while a porpoise showed avoidance behaviour. However, some of these differences may be attributed to the differences in hearing sensitivity within the respective frequency band (approx. 20 dB difference in hearing sensitivity under quiet conditions and an estimated difference in detection thresholds of 5 dB under the background noise in the test pen).

The lessons that can be learnt from pinger studies concerning the development of seal deterring devices are:

- Bycatch reduction in some wild populations may be stable over years, however, some captive studies using pinger-type sounds have demonstrated quick habituation. This shows that with respect to acoustic predator deterrence habituation needs to be considered as a potentially relevant factor.
- In order to minimise any aversive effects on harbour porpoises, sounds produced by seal scaring devices should neither contain harmonics in the frequency band between 20 kHz and 150 kHz, nor solely consist of broadband pulses with substantial energy above 10 kHz. Ideally there should be no energy above 10-20 kHz at all.

Problems and potential solutions

The problem of impact on cetaceans: frequency bands

One of the main differences between the hearing systems of pinnipeds and odontocetes is that the latter are much more sensitive to frequencies higher than 5-10 kHz (see fig 1). In humans the contours of perceived equal loudness follow roughly the hearing threshold in the most sensitive frequency range but contours

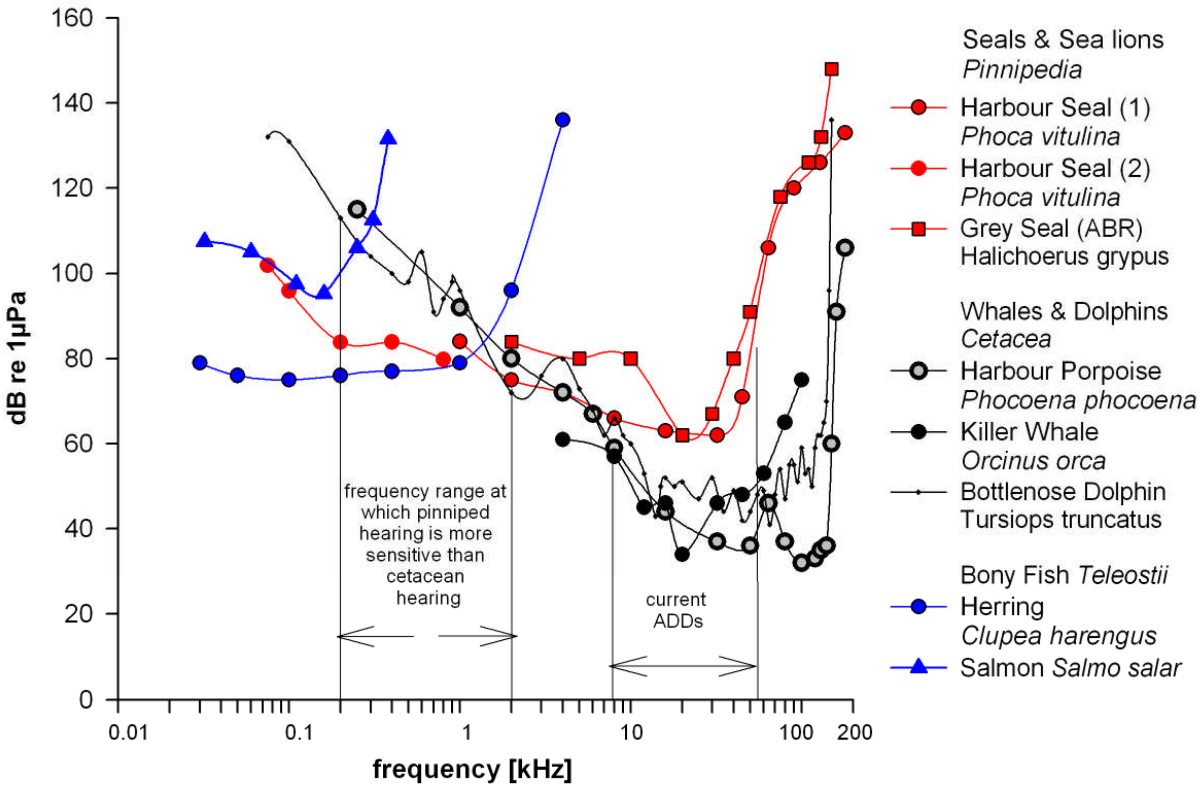

Fig. 1: Hearing thresholds for selected fish, pinniped and cetacean species. Note that most current ADDs operate in a frequency range at which cetacean hearing (dark lines) is more sensitive than pinniped hearing (red lines). Harbour seal (1): Kastak & Schusterman (1998); harbour seal (2): Terhune (1988); grey seal: Ridgway & Joyce (1975), harbour porpoise: Kastelein *et al.* (2002), killer whale: Szymanski *et al.* (1999), bottlenose dolphin: Johnson (1967); herring: Enger (1967), salmon: Hawkins & Johnstone (1978)

flatten towards the edge of the hearing range (Fletcher & Munson, 1933). This means that sounds that exceed the hearing threshold by a similar amount and therefore have similar sensation level are roughly perceived as similarly loud. Fig. 1 shows the hearing thresholds for a representative spectrum of marine wildlife. *Odontocete* hearing is generally 15-30 dB more sensitive than pinniped hearing at frequencies above 4-5 kHz which means that they can be expected to perceive sounds of the same physical sound pressure level as much louder. This coincides with the frequency range at which most of the current ADDs operate. For example, at 10 kHz (the frequency used by the Airmar dB Plus II device) hearing thresholds of cetaceans are 15-20 dB lower than those of pinnipeds (note that 10 dB approximately equals a doubling of perceived loudness). This is supported by recent data that tested the impact of a new acoustic data transmission system on captive harbour seals and harbour porpoises. Kastelein *et al.* (2005, 2006b) tried to determine avoidance thresholds (which they called discomfort thresholds) for both species defined as the source level at the boundary of the area which the animals generally avoided during sound exposure. When averaging these discomfort thresholds for all sound types per species, harbour seals would have an app. 5 dB lower value than harbour porpoises. This is about the difference between the hearing thresholds of both species in the relevant frequencies (12 kHz). Kastelein *et al.* (1995) tested harbour porpoise responses to different sound types. While constant-frequency signals (2.5 kHz) without any harmonics did not elicit a strong reaction, the same signal with a prominent harmonic at 11 kHz did cause the porpoises to exhibit fast swimming behaviour very close to the walls of the pool (interpreted as fright reaction). It remains unclear whether this strong effect is due to the presence of higher harmonics in the signals per se or due to the fact that these harmonics fall into a much more sensitive hearing range of the harbour porpoise. Additionally, the animals did not echolocate during the first trials. Teilmann *et al.* (2006) found similar effects on echolocation activity in two harbour porpoises, however, both individuals habituated quickly.

Therefore, although some of the current ADDs (e.g. Ferranti-Thomson devices) are operating at frequencies close to the most sensitive hearing of pinnipeds (20-30 kHz) these frequencies cannot be generally recommended because thresholds in odontocetes are even lower. Many cetaceans have their most sensitive hearing in the ultrasonic range between 30 and 50 kHz (see Fig. 1). Therefore, if impact on odontocetes is to be mitigated an ADD should not produce substantial energy above 5 kHz. However, a quick glance at Table 1 shows that this is the case for most of the ADD’s that are currently available.

The audiograms of fish species with no specific adaptations general show a rapid decline in sensitivity at frequencies above 500-1000 Hz (e.g. see salmon in fig 1 for an extreme case with almost no high-frequency hearing). However, there are some hearing specialists (e.g. *clupeids*) that have thresholds similar to marine mammals at frequencies of about 1 kHz (achieved by a coupling of the swim-bladder with the sound-sensitive organ). Generally speaking, a frequency band between 700 Hz and 2 kHz for ADDs would be ideal in terms of target-specificity in the context of mitigating impact on odontocetes. Compared to the frequency band used in current seal scarers this would lower sensation levels in odontocetes by about 40 dB which should lead to a dramatic reduction in deterrence ranges. One problem might be that fish species with good high-frequency hearing could be affected. Most fish species with specialized hearing (e.g. herring; see fig 1) are pelagic animals and therefore less likely to be in the vicinity of a fish farm but coastal spawning grounds would have to be taken into account and should be considered on a case to case basis. American shad *(Alosa sappidissima)* also occur in coastal waters and have been shown have a broad hearing range extending up to 180 kHz. However, the absolute hearing sensitivity of shad at frequencies between 1 and 2 kHz is rather low (ca. 130 dB re 1µPa) and the species is 30-40 dB less sensitive than most odontocetes at these frequencies. Marine mammals that use low-frequency sounds for communication and for which no audiograms are known (e.g. baleen whales) would also need to be considered. Therefore, potential impact on both hearing generalists and specialists among fish and baleen whales should be assessed before using this frequency band. Also, low-frequencies are attenuated less strongly by absorption and can therefore affect a larger area around a fish farm.

The problem of loudness perception: source levels

Most examples in this section are based on human sound perception since no data are available for marine mammals. Although there are specific adaptations to aquatic hearing there is currently no evidence that the basic functioning of the cochlea and peripheral auditory processing in the brain is substantially different in marine mammals. The general paradigm applied in current ADDs is that a high source level sound is expected to cause physical discomfort or even pain and therefore results in the animal leaving an area. There are several problems involved when operating at the upper end of the dynamic range of an animal. The relationship between the

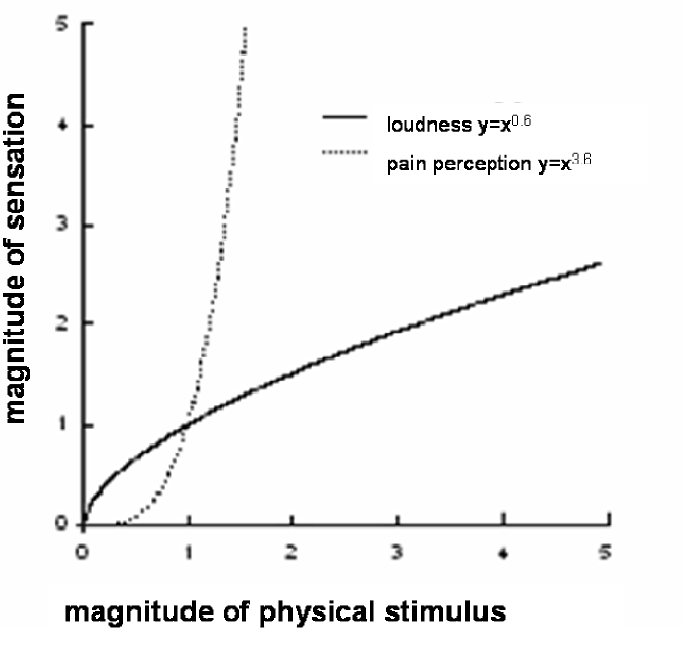

Fig 2: Qualitative representation of Steven’s law (arbitrary units)

magnitude of sensation (Ψ) and the magnitude of the physical parameter (φ) can be approximately modelled by Stevens' law (Stevens 1956):

$$\Psi = k\,(\varphi-\varphi_0)^m$$

with k being a constant, φ_0 being the lowest perceivable physical stimulus (threshold) and m being a modality-specific coefficient determining the essential shape of the function. In the human auditory system m=0.6, however, other sensory modalities have been found to have exponents higher than one e.g. m=3.6 for pain caused by electric shocks (see also fig 2 and Stevens 1961). Therefore in the auditory system a given increase of the level of a high sound pressure stimulus leads only to a small increase of the perceived loudness, while the same increase of a low sound pressure stimulus would lead to a stronger increase in perceived loudness (see fig.2).In this context "increase" does not refer to a ratio but means adding a defined sound pressure value. The increase of loudness in terms of a sound pressure ratio as

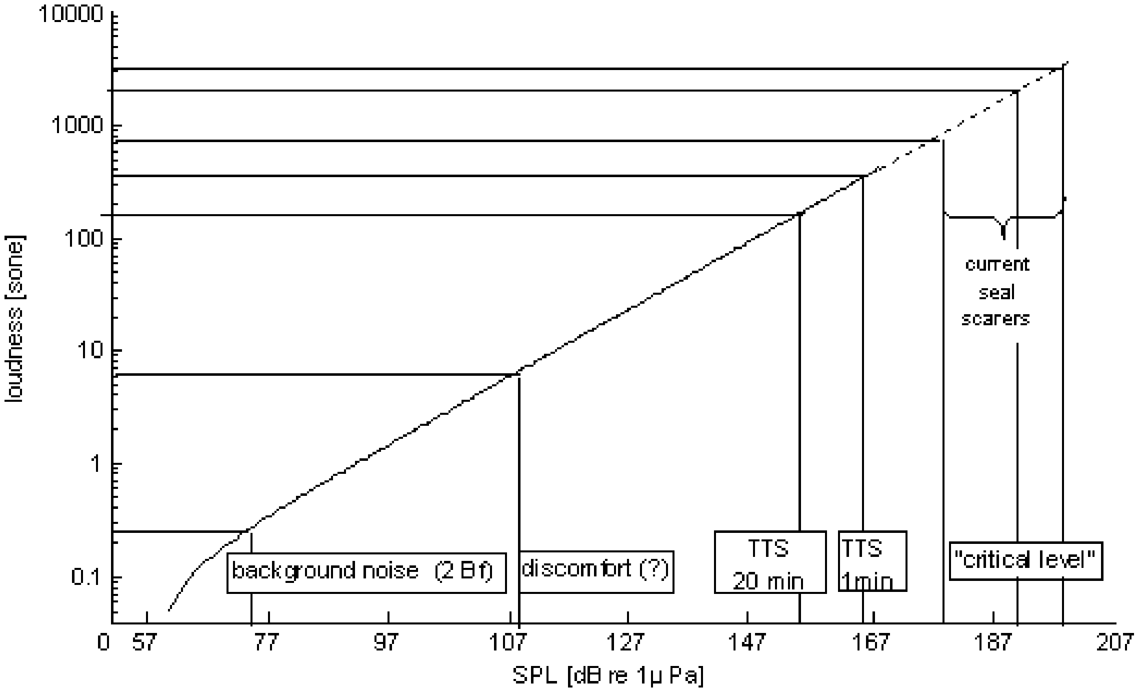

Fig. 3: Loudness scale for the harbour seal. The y-axis shows the perceived loudness in sones.; a doubling of the loudness in sones reflects a doubling in perceived loudness. The x-axis represents the sound pressure level with 57 as the hearing threshold at 2.5 kHz (Kastak *et al.* 2005). TTS means that the source level causes a temporary threshold shift if the animal is exposed for the mentioned amount of time (based on SEL measured by Kastak *et al.* 2005). Values for current seal scarers are source levels at 1m distance. The discomfort threshold is taken from Kastelein *et al.* (2006), critical levels are based on data review in section on hearing damage.

reflected by the dB scale is in fact constant. Thus, an increase of sound pressure in the upper range of the curve in fig. 2 can be expected to disproportionately increase the risk of damaging the ear without yielding a much stronger aversive effect. The perceived loudness of a sound is generally measured using the sone scale. On the sone scale a doubling does reflect a doubling of perceived loudness. One sone means that a sound has a perceived loudness equal to that of a 40 dB re 20 µPa tone at 1 kHz in air for humans. 2 sone would then mean that a human subject judges another stimulus as twice as loud as the original 40 dB re 20 µPa tone. The perceived loudness in sone (L) can be calculated by the equation $L=0.01\ (p-p_o)^{-0.6}$ where p is the sound pressure in µPa and p_o is the effective hearing threshold (Scharf 1978). Fig. 3 applies Steven's law to the harbour seal's hearing threshold and shows different sound sources on a sound pressure level/perception scale. The "discomfort" threshold based on the avoidance responses for harbour seal described on Kastelein *et al.* (2006 b) would be at about 6 sone which is slightly lower than what has been reported for human subjects (see Fig. 3). Pain thresholds are much higher and usually similar to sound pressure levels that cause immediate hearing damage (see Spreng 1975 for pain and discomfort thresholds). This means that current seal scarers are not likely to cause "pain" or if they do (as some manufacturers claim) they are also likely to cause hearing damage.

Apart from immediate damage long-term exposure to moderate levels can also lead to permanent damage. This can easily occur without any pain. For example, students working in entertainment venues have been shown to have permanent threshold shifts up to a maximum of 30 dB (Sadhra *et al.* 2002). Therefore, no attempt should be made to increase the source level currently used or, indeed, use devices that emit sound continuously at source levels that fall at the upper end of the dynamic range close to the suspected pain threshold. Additionally, the critical level of about 135 dB above the threshold should not be exceeded at reasonable distances from the sound source since several studies showed that the risk of damage originating from single, short-term exposures is substantially increased above this level (Danielson *et al.* 1991; Levine *et al.* 1998). A safe exposure level for seals would be a perceived sound exposure level of about 126 Pa^2-s above the threshold which equals a SEL of 183 dB re 1 μPa^2-s (meaning exposure of 183 dB re 1µPa for up to 1 s). This was calculated for a 2.5 kHz tone played to a harbour seal (Kastak *et al.* 2005). Recovery times in sound exposure scenarios that do not cause TTS should be at least 10 s to avoid accumulation of acoustic trauma (Kryter 1985). Longer or higher intensity sound could be used if a seal scarer would stay switched off for the time required for recovery from TTS (which can range from minutes to days, depending on the amount of TTS caused and is a function of exposure level and time). Since cetaceans have a higher hearing threshold in the suggested frequency band of 500 -2000 Hz the effect on these animals should be less severe than with existing ADD’s. However, if conventional seal scarer sounds are to be used the acceptable exposure levels should be based on hearing thresholds for each potentially affected species.

How do seals perceive sound ?

Factors other than loudness could possibly be used for deterring seals. Zwicker & Fastl (1990) developed a model to describe what makes sound pleasant or unpleasant for humans. The relevant psychophysical parameters in the model are sharpness, roughness, tonality and loudness. The perceived pleasantness of a sound may be based on the general functioning of the periphery of the mammalian auditory system (e.g. the cochlea) and it may therefore be worthwhile to test whether animals’ judge sounds in the same way as humans. A two-alternative forced-choice experiment in rats revealed preferences for musical consonances (Borchgrevink 1975), a phenomenon that was believed to be a result of culture in humans. However, preference experiments with nonhuman primates failed to find such evidence (McDermott & Hauser 2004).

How to prevent habituation ?

Motivational factors are very likely to influence responses to sound exposure. For example, playbacks with harbour seals in a pool resulted in an exclusion of the animals from a zone with exposure levels higher than 108 re 1µPa without any habituation in 7 consecutive playback sessions per sound type (Kastelein *et al.* 2006 b). The fact that seals predating on fish farms appear to tolerate much higher exposure levels shows that food motivation has a major influence on deterrence.

A triggering method that only plays sounds when seals approach could help to postpone habituation (see Ace-Aquatec www.aceaquatec.com). Additionally, several manufacturers claim that using highly variable sound types prevents habituation (e.g. Terecos ltd). However, no empirical data for animals in a feeding context are available to support this claim. Habituation to acoustic stimuli has been studied extensively using the acoustic startle response (ASR) in rodents (e.g. Moyer, 1963).

The startle response is elicited through a relatively simple reflex that became a model system for studying the neural basis of basic learning processes like sensitisation and habituation (Koch & Schnitzler, 1997). According to Pilz & Schnitzler (1996) habituation in the startle pathway is not caused by an increase of the threshold eliciting the response, but by a change of the slope of the input (SPL of signal)-output (magnitude of response) function. The authors suggest that this provides evidence for the dual process theory of habituation meaning that the response to a repeated stimulus is influenced by a decreasing (sensitisation) and increasing (habituation) component (Groves & Thompson 1970). In terms of an application in a seal scarer this means that one would ideally enhance the sensitising components of the process or even better replace habituation by sensitization to a sound stimulus. In rodents presentations of intense sound pulses or electric foot-shocks (500ms, 0.4. ms rise-decay time, 119 dB re 20µPa) have been shown to cause sensitisation to lower amplitude acoustic stimuli eliciting startle responses (Plappert et al. 1999). This paradigm could probably be implemented in seal scarers by using high intensity sound stimuli intermittently to sensitize a lower-intensity stimulus. Sensitization through electric stimulation would be difficult to implement underwater because it would most likely impact the fish in the pens.

It is likely though that trying to prevent habituation or extinction of an aversive behaviour will not be possible unless a stimulus has negatively reinforcing properties (see Skinner, 1996 and Pryor 1987 for marine mammals). It is possible that some acoustic stimuli e.g. sound pulses that elicit an evolutionarily old reflex arc like the startle response might act as an unconditioned stimulus with reinforcing properties but this has not been tested yet. It may also be possible to apply classical conditioning paradigms e.g. in the following way: an unconditioned stimulus (UCS) e.g. a fish treated with a substance that causes sickness (emetic) or maybe even a startling sound causing an unconditioned response (UCR; avoidance) is associated with a conditioned stimulus (CS) e.g. an artificial acoustic signal with no biological meaning. After several pairings the CS is able to cause the conditioned response (CR) which consists of the same behavioural pattern as the unconditioned response. Unfortunately, reinforcement methods are limited in an underwater environment and the only known way is to use emetics (e.g. ivory soap, LiCl). Emetics have been shown to be temporarily successful against California sea lions (Kuljis 1984; Costa 1986) but some animals learnt to avoid treated fish after a while and continued to feed on the natural salmon run (National Marine Fisheries Service 1996).

Unfortunately, pairing of food aversion learning with non-gustatory modalities (e.g. sound) does not seem to work very effectively (Nachman & Ashe 1977).

Potential use of biological sound

A classical approach for acoustic deterrence is the playback of predator calls. For pinnipeds this would mean to use playbacks of killer whales calls since no other vocalizing predator is known. Killer whales produce clicks (broadband), whistles (tonal) and calls (Ford & Fisher, 1982). Akamatsu et al. (1996) conducted playback experiments exposing captive Steller Sea Lions to killer whale calls. Avoidance responses declined rapidly after the first trial and no effects on adult males could be observed. Fish & Vania (1971) found a sudden avoidance reaction in migrating white whales exposed to killer whale calls (source level 170 dB re 1µPa) in several consecutive trials. Cummings & Thompson (1971) used killer whale calls in a similar way and described the responses of grey whales (*Echrichtius robustus*) as being “dramatical”. The animals retreated immediately, reduced their blows and wakes when surfacing, spy-hopped and nearly stopped vocalising completely. Cummings & Thompson (1971) also tested tonal sounds resembling the main components of killer whale calls and noise, both yielding only little responses. Deecke et al. (2002) showed that the number of seal sightings only dropped significantly when sounds of a transient killer whale population that mainly fed on marine mammals were played while no effect was caused by calls from fish-eating killer whales which the authors interpreted as selective habituation to calls from non-mammal eating populations. In conclusion, there is a possibility that killer whale calls could be an effective deterrent, however, in the light of findings by Deecke et *al.* (2002) one would expect seals to habituate. Most importantly, responses described in cetaceans show that playback of killer whale calls would not be target-specific. This approach has therefore to be disregarded for most applications.

A target specific method for pinnipeds could be to use playbacks of consepcific calls since they should not influence odontocetes or other cetaceans. Van Parijs et al. (2000b) found that harbour seal vocalisations of individual males are highly clustered in discrete areas (separated by 200-250 m) of a similar size (40 and 135 m^2). Display areas are maintained in several consecutive years which clearly indicate territorial behaviour. Nicholson (2000) showed that dominance hierarchies in harbour seals are established by repeated confrontation (e.g. surface splashing displays, calling) with dominant males producing long, low-frequency (LL) roars. Hayes et al. (2004b) tested the function of these sounds in a playback experiment. They hypothesized that roars serve intra- and inter-sexual function and indicate male quality through acoustical features. Seals approached the loudspeaker in 62.5 % of the sessions in which short high-frequency (SH) roars were played, but only in 25% of the LL sessions (0% in control with white noise). The playback position was changed between trials and only one male responded at each position. Females seemed not to be influenced supporting the idea that roars are a territorial signal. These experiments gave evidence for a role in male-male competition. Since the experiment yielded no clear evidence for any responses in females it is questionable if roars also serve to attract females. Hayes et al.'s (2004b) findings could have several implications for developing new deterring sounds. If specific features of a harbour seal roar would be exaggerated (lowering the frequency and increasing duration) it might be possible to cause an avoidance reaction in all male seals.

While mating in grey seals has been regarded as solely terrestrial, Lidgard et al. (2004) found that although reproductive success in males forcing a copulation underwater is lower, it still represents a relatively successful strategy. Therefore, male grey seals may also use underwater vocalisations for displaying their traits. Asselin (1993) recorded grey seal sounds throughout the mating season and found a variety of sounds to be used with "rups" (48%) being the most common sound type. This vocalisation consists of a low-frequency onset between 100 and 300 Hz and a sharp noisy upsweep up to 4.7 kHz. An analysis of Scottish video footage showing a male grey seal attempting to copulate underwater indicated that the predominant vocalisation was similar to Asselin et al's. (1993) "rups" (McCulloch 1999). Another vocalisation of interest could be the growl having fundamental frequencies between 100 and 300 Hz with no or some noisy harmonics. It seems to be associated with dominance and mating (Schneider, 1974). Playback of conspecific calls for acoustic deterrence therefore needs careful investigation since calls could be attractive as well as aversive.

Discussion

Efficiency of acoustic deterrent devices differed hugely between studies and study sites which might be the result of differences in environmental conditions, seal populations and deployment. Alternatively, differences could be an artefact of study design. Generally speaking, efficiency seems to range from poor to moderately effective with only a few examples where ADDs seem to be very effective.

Habituation to ADDs occurred within varying time frames ranging from days to years but it seems to be a substantial problem in almost all cases. In contrast, non-target species have been shown to be excluded from their habitat and long-term studies did not find any obvious habituation of these effects. This is most likely because odontocetes have more sensitive hearing in the devices' frequency range and apparently little motivation to feed on farmed fish. Therefore, in order to increase target-specificity ADDs should use signals that fall in a frequency band between 700 Hz and 2 kHz (ideally with most energy centred around 1 kHz). However, potential impacts on hearing specialists among fish and baleen whales should be investigated if these occur in the vicinity of the fish farm. In no case should ADD signals contain much energy above 5-10 kHz if odontocetes have an important habitat around the fish farm. Additionally, methods to reduce the duty cycle should be found e.g. the effects of triggering methods or presentation of isolated sound pulses should be more thoroughly tested. When triggering methods are used signals should be short to avoid hearing damage and the maximum sound pressure level should be chosen based on available data for the onset of temporary threshold shifts.

Most current, commercially available ADDs may have some potential to damage the hearing of marine mammals, particularly if an animal stays in the vicinity of a fish farm and is exposed to sound emissions repeatedly. It should also be considered that in areas with a high density of fish farms acoustic trauma may accumulate similar to exposure of workers to industrial noise. Therefore, only sound exposure protocols that use sound pressure level exposure-time combinations which result in a sound exposure levels (SEL) that do not cause hearing damage in pinnipeds and cetaceans should be used. Methods to prevent habituation such as fear conditioning deserve further study. Furthermore, since food motivation may be a crucial point, any newly established fish farm should try to prevent predation from the beginning. If several seals have already started predation there is only little chance of success of deterring seals with any acoustic method. Most importantly, efficiency and target-specificity of any device should be tested by independent studies before devices are deployed in fisheries or on fish farms.

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Chapter 3

Aversiveness of artificial sounds: Behavioural responses depend on psycho-physiology and motivational state

Introduction

Behavioural responses to sounds that have no biological meaning are likely to be influenced by psycho-physiological factors and motivational processes. On the perceptual side, perceived loudness and pleasantness are of crucial importance. As mentioned in chapter 2 cetacean and seal hearing is not equally sensitive over a range of different frequencies. Psychophysical experiments on humans showed that the contours of perceived equal loudness are roughly parallel to the hearing threshold within the most sensitive hearing range but are compressed at the high and low frequency edge of the hearing range (Robinson & Dadson, 1956; Fletcher & Munson, 1933). A rough approximation is therefore to assume that sound pressure levels that exceed the hearing threshold by a similar amount cause similar perceived loudness. These so called sensation levels are expressed as sound pressure level in dB above the hearing threshold. While perceived loudness depends in part on stimulus amplitude it is important to note that the physical composition of a sound does also contribute to perceived loudness (Fletcher & Munson 1933). For instance, the perceived loudness of a group of pure tones or filtered noise increases rapidly if the bandwidth of a stimulus exceeds the cochlear filter bandwidth (critical bandwidth) at a given frequency while the source level is being kept constant. In contrast perceived loudness remains almost constant if the bandwidth of the stimulus stays within a critical band (Zwicker et al., 1957). In addition duration of a sound influences its loudness: for stimuli close to the auditory threshold perceived loudness increases with increasing stimulus duration up to a maximum of 200ms. For louder sounds a continuous increase up to a duration of 100ms was found (Zwislocki, 1969).

Perceived pleasantness has mainly been studied in humans. Here, a variety of psycho-physical features of a sound influence its pleasantness. Zwicker & Fastl (1990) developed a model to describe what makes sound pleasant or unpleasant for humans. The relevant psychophysical parameters are sharpness, roughness, tonality and loudness. Roughness can be maximised by using strong frequency or amplitude modulation with maximum roughness caused by modulation frequencies of about 70 Hz and frequency modulation contributing more to roughness than amplitude modulation (Zwicker & Fastl, 1990). Interestingly, high loudness contributes with the lowest loading to unpleasantness for low and medium–intensity stimuli. Sharpness is mainly correlated with higher centre-frequencies within the species hearing range and tonality depends on the waveform of the sound being highest for pure-tones and low for square wave sounds. Furthermore in humans complex sounds that consist of partial tones that are related by certain frequency ratios (musical intervals) are perceived as unpleasant (dissonant) while others are perceived as pleasant (consonant). Modern classical composers (e.g. Arnold Schönberg) assumed that this is a result of culture but physiologists like von Hemholtz (1853) expected more general properties of the auditory system to be responsible. Von Helmholtz (1853) hypothesised that consonance depends on how many harmonics of two complex tones match. Non-matching harmonics result in “beating” phenomena causing a sensation of roughness. In spite of strong criticism e.g. by musical psychologists (Stumpf, 1883) consecutive research gave support for Helmholtz’s notion that interference of adjacent partials is important (see Plomp, 1965). Plomp & Levelt (1965) showed that in musically untrained subjects transition from consonance to dissonance perception depends on the cochlear filter bandwidth (critical bandwidth). The strongest perception of this so called “tonal dissonance” is caused by two partials that fall within 25 % of the critical bandwidth. This critical band theory can also explain roughness perception based on frequency or amplitude modulation (Zwicker & Fastl 1990). Given that the phenomenon of unpleasantness seems to be associated with factors as basic as auditory filter bandwidth one would expect animals to have similar sensations. However, experimental evidence, at least for musical consonance perception in animals is still equivocal. A two-alternative-forced choice experiment revealed clear preferences for consonant musical intervals in rats (Borchgrevink, 1975). However, neither consonance preference nor preference of white noise over “screeching sounds” was found in place preference experiments with monkeys (McDermott & Hauser, 2004). Further experiments on non-human primates revealed preference for slow tempos over fast but also a general dislike of music (McDermott & Hauser, 2007). In contrast experiments on Japanese song sparrows (*Padda oryzivora*) gave limited evidence that some birds which preferred music over silence in a first experiment also showed preference for tonal music (e.g. Bach) compared to modern atonal music (e.g. Schönberg) (Watanabe, 1998). Although one might assume that atonal music contained more dissonant intervals, music preference experiments in animals are notoriously difficult to interpret because it is impossible to discern the influence of different features in sounds that are as complex as music.

Motivational factors influence behavioural responses to artificial sounds on another level. Although most reviews on the impact of noise on marine mammals mention motivational state as a potentially important factor influencing behavioural responses to sound (Richardson et al., 1995; Southall et al., 2008; Nowacek et al., 2007), no systematic experimental study attempting to discern different factors has been carried out so far. However, anecdotal evidence indicates an important role of motivation. Such evidence comes from studies that show a decreasing (Mate & Harvey, 1987) or absent effectiveness (Norberg, 1998) of acoustic deterrent devices in situations where food motivation was high.

All of these findings are also relevant for the choice of a stimulus in acoustic deterrent devices if the declared aim is to use the minimum source level that is required to elicit avoidance responses in order to minimise noise pollution and impacts on hearing (as suggested in chapter 2). Most commercially available acoustic deterrent devices use high source levels at high duty cycles (up to 50%) which are expected to cause pain if seals approach too closely (see Jeffers 1996, US Patent No. 5610876). In humans the pain threshold lies at around 120 dB re 20 µPa (Spreng, 1975) and is therefore quite close to sensation levels where short, single exposure can damage the ears of a terrestrial mammal (130 dB re 20 µPa, Henderson, 1991). At close ranges these sounds would also exceed recommended maximum sensation levels for humans if exposure lasted more than 1.5min per day (Kryter, 1994). Therefore, if these considerations about hearing damage are also true for seals then acoustic deterrent devices should ideally not produce received levels that cause pain. High duty-cycle devices operating close to the pain threshold would be particularly problematic since longer exposure will increase the risk of hearing damage (see chapter 2). However, discomfort or distress thresholds may be lower and may be used to cause a deterrence effect. Spreng (1975) showed that in humans, changes in electro-physiologically measurable parameters that are indicative of discomfort and stress occur at sensation levels as low as 70-80 dB above the hearing threshold. In the light of these considerations the sound of a high duty-cycle ADD should be below the pain threshold to avoid potential hearing damage but it should be above the discomfort threshold to cause a deterrence effect. If discomfort thresholds in seals and humans are similar (70-80dB above hearing threshold) then an 8 kHz ADD would have to produce a received levels that exceeds 134-144 dB re 1 µPa within the designated deterrence zone (hearing threshold from composite audiogram; see appendix 1) The biggest problems with current ADDs is that they have dramatic effects on non-target species e.g. habitat exclusion of *odontocetes* was shown up to distances of over a kilometre from the sound source (see review in chapter 2) When attempting to mitigate impact of ADDs a frequency band should be chosen where hearing in *odontocetes* is less sensitive than hearing in seals. As it was argued in chapter 2 this would be the case for frequencies between 500Hz and 2 kHz. Harbour porpoises (*Phocena phocoena*) have been shown to avoid an area of 645m around a commercial ADD (10kHz) which corresponds to modelled received levels of 128 dB re 1µPa (Johnston, 2002). Assuming the hearing threshold of a harbour porpoise to be 50 dB re 1µPa at 10 kHz (Kastelein et al., 2002) the received level equals a sensation level of 78 dB. Given that the harbour porpoise' hearing threshold is at least about 30dB higher at 1 kHz received levels causing a deterrence effect can be expected to be in the order 158 dB re 1µ Pa. Assuming simple spherical spreading such a received level would be reached at 15m distance. This would mean that theoretically using the suggested frequency band could result in a dramatic reduction of deterrence ranges for *odontocetes*.

My study aimed to test how different factors related to psycho-physiology or motivation influence behavioural responses to noise in *phocid* seals. This was done by testing responses 1.) "new sounds" based on current psychophysical knowledge of what makes sounds unpleasant to humans 2.) sounds from commercially available ADDs ("seal scarers"), 3.) control sounds with assumed neutral properties. To test how motivation modifies behavioural responses to noise seals were tested in 3 different situations with a) a profitable food source b) a known but "exploited" food source c) no food source. The "new sounds" were also based on the suggested frequency band to mitigate impact on *odontocetes*. Zwicker and Fastl's (1990) model was used to design the supposedly "unpleasant" sounds e.g. by applying strong frequency modulation to increase "roughness". Therefore, the data were also expected to possibly shed some light on the perceptual (or alternatively cultural) basis of "pleasantness" of sounds in humans.

Experiment 1: Responses of wild captured seals in captivity under different levels of food motivation

Methods

Subjects and pool

Six grey seals (*Halichoerus grypus*) and two harbour seals (*Phoca vitulina*) were tested in the experimental facility of the Sea Mammal Research Unit at the University of St Andrews (Scotland/UK). All seals were wild-captured at the haulout-site at Abertay Sands (56^0 25.59’ N, 2^0 45.59’ W) approximately 10km north of St Andrews (Scotland/U.K.) with the exception of one of the male harbour seal that was caught in the Eden Estuary 3 km east of Guardbridge in Scotland / UK (approx. 56^0 21.7’ N, 2^0 51’ W). Seals were captured by hand in hoop-nets after a fast boat approach of the haulout. One harbour seal was captured using a seine net. The captured seals were restrained in pole-nets and taken to the facility of the Sea Mammal Research Unit in St Andrews. Seals were housed in outdoor pools filled with sea water and fed a diet of mackerel, *Scomber scombrus*, haddock, *Melanogrammus aeglefinus* and herring, *Clupea harrengus* and sprat, *Sprattus sprattus*. All animal handling procedures were approved by the University of St Andrews Ethics committee and carried out in accordance with Home Office regulations.

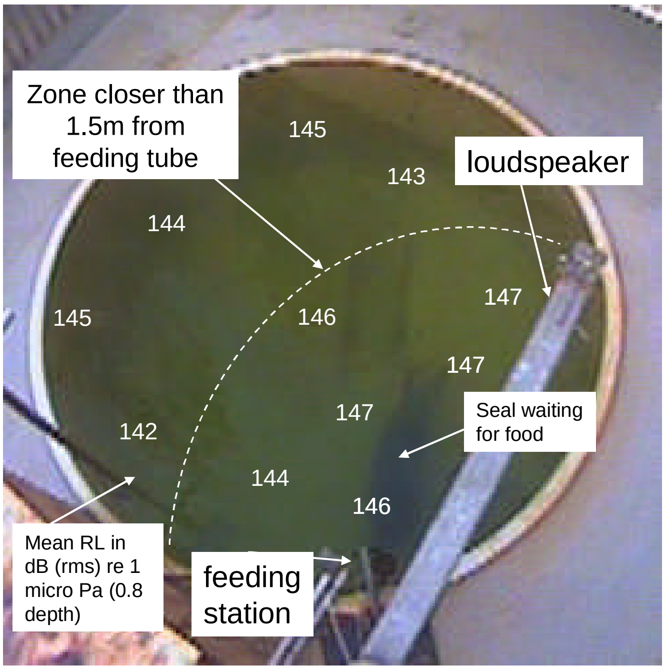


Figure 1: Experimental setup and sound field in playback pool

Four out of the 6 grey seals were sexually mature adult females and 2 were juveniles (one male, one female). Juveniles ranged in age from approximately 6-11 months at the time of the experiments. The two harbour seals were adult males. One of the male harbour seals had been flipper tagged in 1999 indicating that it was at least 8-

10 years old. The harbour seals were in the facility for two weeks and one month respectively before being used in the experiment while the tested grey seals were in the facility for a time ranging from 3-8 months prior to the experiments. Experiments were carried out in a 3-m diameter, 1.5 m deep circular, sea-water filled pool. The pump that was circulating seawater through the pool was switched off for experimental periods.

Playback stimuli

All playback sounds were synthesized using Cool Edit pro software and normalised to the same average rms-level.

The artificial sounds (new sounds) based on the Zwicker & Fastl (1990) model for pleasantness to maximise unpleasantness in the experiments were:

- Square 500/530 FM: Two 70-Hz frequency modulated square-wave tones with a carrier frequency of 500 and 527 Hz were synthesized (referred to as partials). Modulation depth was 50 % of the carrier frequency. The frequency ratio between these two partials was chosen to reflect the musical interval of a minor second. Then both partials were mixed (overlaid). The stimulus can therefore be expected to be aversive because of the roughness caused by the frequency modulation in addition to a possible effect caused by the two partials falling roughly into 25% of the critical bandwidth (similar to the musical interval of a minor second). Directly measured critical bandwidths in air using band-narrowing techniques have been shown to range mostly from 20-40% of the test frequency in pinnipeds (Southall et al., 2003).
- Square 500/507 FM. The stimulus was synthesised identical to “Square 500/530 FM” except for that the carrier frequencies of the two partials was 500 and 507 Hz respectively. The frequency ratio of the partials for this stimulus was chosen to reflect 25% of the critical bandwidth calculated from underwater critical ratios in pinnipeds (Southall et al., 2000). Critical bandwidths calculated from underwater critical ratios were found to be between 3% and 9% of the test frequencies. This alternative approach was used to account for uncertainty in the data on pinnipeds critical bandwidths
- Square FM: 70 Hz-frequency modulated square-wave tones with a carrier frequency of 500 Hz. Modulation depth was 50 % of the carrier frequency
- Sweeps FM: This was a complex sound consisting of frequency modulated square wave up- and down-sweeps. The frequency modulation applied to the

square waves ranged from 0 (no modulation) to 100 Hz with modulation depth between 30 and 60 %. The sweep range covered a frequency band from 400 Hz up to 3.5 kHz and sweeps duration ranged from 2-4 s. Average peak frequencies of the broadcasted sound ranged from 750 Hz to 1.5 kHz.

- Square variable: This sound consisted of short (100 ms to 300 ms) constant-frequency square wave pulses. The carrier frequency of each individual pulse ranged from 500 Hz to 1.5 kHz. Some of the square wave pulses were frequency-modulated similar to sound type “Square FM”.

In spite of attempts to compensate for the low-frequency decline in the response of the loudspeaker, the actual peak frequencies of all five new sounds broadcasted through the loudspeaker were between 750 Hz and 800 Hz. The -20 dB power points were between 600 Hz and 2.5-3.5 kHz respectively.

Recorded sounds of commercially available seal scarers:

- Airmar dB Plus (recorded during an acoustic survey; provided by C. Embling): Pulse train consisting of short pure tones (10 kHz)
- Terecos Ldt. (recorded during an acoustic survey; provided by C. Embling): Complex, broadband sounds; peak frequency of audible component between 7 and 9 kHz
- Ace-Aquatec/ Ferranti-Thomson seal scarer (own recording using B&K 8103 hydrophone and B&K 2346 charge amplifier): Short tone pulses at varying frequencies; peak frequency of audible components either around 15.4 kHz or 9.6 kHz
- Lofitech (this device produces a 11 kHz sine wave; the sound was synthesized based on features extracted from a recording provided by M. Wahlberg; the original recording was not used because of strong surface reflections)

Control sounds:

- White noise (up to 24 kHz)
- Sine wave (500Hz)

Transducer, sound field and source level

Sounds were played through a Lubell 9162 loudspeaker (Lubell Labs Inc, Columbus, Ohio) that was placed 1m away from an underwater feeding station hanging freely from a crane approximately 20cm from the wall of the pool. The loudspeaker was powered by a Phonic MAR 2 power amplifier and playback sounds were played from a Panasonic SL-S120 CD player. A preliminary transducer calibration was carried out from a boat moored in St Andrews harbour in 2 m deep water with the hydrophone and loudspeaker being separated by 2 m. Sine wave tone, sweeps, white noise and all playback stimuli were played at source levels ranging from 120 to 160 dB re 1µPa in order to estimate the frequency response of the loudspeaker. All sound types that contained significant energy below 700 Hz were equalized using the calibration data to compensate for the low-frequency response decline (<800 Hz) of the transducer using FFT-filters in Cool Edit pro. Additionally, the amplitude of some playback stimuli had to be adjusted digitally to ensure an identical source level for all stimuli. The loudspeaker was then placed at the correct position in the pool (see figure 1) and received levels of all playback stimuli were measured 4 times at 11 different positions of the pool. The average received level at the typical position of the seals' head was 146-147 dB re 1 µPa (rms). Assuming the hearing threshold of harbour seal to be 72 dB re at 1 µPa at 1 kHz (see composite audiogram in appendix 1) these sounds would have a sensation level of 74 dB and therefore exceed the discomfort threshold in humans. The lowest average received level measured in the pool was 142 dB re 1 µPa (rms); the highest was 147 dB re 1 µPa (rms).

Transducer calibration and sound field measurements were done using a calibrated Bruehl & Kjaer 8103 hydrophone connected to a Bruehl & Kjaer charge amplifier 2635 operating in acceleration mode. The output from the charge amplifier was recorded through the line-in of a Toshiba Satellite Pro laptop with the in built sound card (SoundMax Digital Audio). The sound card was calibrated using a Thurlby Thandar TG 230 signal generator. The output from the signal generator was confirmed with a Tektronix TDS 3022 digital oscilloscope capable of doing accurate peak-to-peak and rms voltage measurements. The sound card showed a flat response (+/- 1.5 dB) in the frequency band from 70 Hz to 15 kHz. The amplification of the sound card was calculated by dividing the actual recorded voltage by the known voltage of the calibration signal from the charge amplifier. The voltage of the calibration signal from the charge amplifier was also verified by measurements with the digital oscilloscope. Recordings were made using Cool Edit Pro 1.2 software (Syntrillum Software Corporation). Root-mean-square (rms) and peak-to-peak (p-p) voltages of the recorded sound and calibration signals were measured in Avisoft SAS Lab Pro v 4.32 (Avisoft Bioacoustics, Raimund Specht, Berlin). The sound pressure (SP) was calculated from the corrected recorded voltage output from the charge amplifier (e.g. when the gain of the charge amplifier is set to 1mV the amplifier will output 1mV per Pa; knowing the gain of the sound card this can be calculated back to sound pressure measurements). Sound pressure levels (SPL) were calculated as SPL=20log (sound pressure/1µPa).

Experimental protocol

Every playback lasted one minute with sound being presented 4 times as a 6 s sound burst. This resulted in an effective duty of 40 % cycle over the 1 min period. The interval between each of the four presentations was therefore 12 s. These one minute playback and observation periods were separated by 5 min pauses. A playback session consisted of playbacks of all 11 described sound stimuli (5 new sounds, 4 seal scarers, 2 control sounds) separated by 5 min breaks and a 1 min observation period with no sound presentation (acting as no sound control). Different examples of the recorded sounds were used in different playback sessions to prevent pseudo-replication. For some later analysis values obtained for a certain response variable were averaged for each of the categories of sound (new sounds, seal scarer, control sound). This was done to enable further statistic tests avoiding the problem of losing too many degrees of freedom by including all 11 sound types. Sounds in each of these categories fall into a similar frequency band (with the exception of white noise and sine wave, however, these were pooled since they were expected to have both neutral properties with respect to “pleasantness”). The term “treatment” is used to refer to exposure to either 1) new sounds 2) control sounds 3) seal scarers 4) no sound.

Experiments were carried out in the following way: A fish in a metal cup was lowered through a tube (feeding station) that had a window at approximately 1-1.2 m depth. As the edge of the cup became visible the animal would position its head in front of the feeding station since the seal knew the food source from previous feeding by using the cup in the tube. If the animal’s tip of the nose was within 40 cm distance of the cup the playback started. The cup was then lowered completely 2 s after the playback onset so that the seal could access the fish. Three playback sessions were carried out with food presentation. Then, one session was conducted in which the playback of each sound started when the seal positioned voluntarily but no food reward was given (no food trials). The fifth playback session was again a food trial while the last one was another no food trial. This was done to test how different motivational states influence behaviour (known but empty food source versus profitable food source). Playbacks were monitored using an HTI-96-MIN hydrophone, an analogue VN37CPH colour underwater camera focused at the feeding station and a second overhead video camera mounted on a 4m long pole that was used to overview the whole pool area. Video tracks from both cameras were linked to a multiplexer and together with the audio track from the hydrophone recorded on either a Sony DV video walkman (GVD 1000E) or on a Sony MVX 350i video camera (through the AV port). The experimenter and all equipment were hidden form the animal in a hut next to the pool. Behavioural responses were measured after the experiment by analysing the video recordings.

The following response variables were continuously recorded from the video tapes during the 1 minute sound exposure:

Index of aversiveness defined as a cumulative index of occurrence of a series of aversive behaviour patterns. Depending on whether all or none of the following patterns occurred the index could range from 0 (not aversive) to 4 (highly aversive):

- fish catch prevented: Fish remains in cup for the whole minute
- seal turns away from speaker: A change in the orientation of the line between shoulder blades and the tip of the noise by at least 100º from the original orientation (nose pointing towards feeding station).
- Escape/Flight response: Seal increases distance to speaker at speeds of more than 3m/s. This behaviour was counted if the animal crossed the pool diagonally swimming away from the feeding station in less than 1 s.
- haul-out behaviour for at least 30 s (after flight response)

Although these 4 types of behaviours are probably not equally severe some can occur independent of each (e.g. fish catch prevented and haulout) and therefore a cumulative index instead of an ordinal scale was chosen.

Additionally the following continuous behavioural response variables were measured:

- Time the animal spent underwater within 1.5 m distance of the feeding tube (position of head was measured)
- dive time during playback (max 1 min) defined as head being completely submerged.
- time hauled out defined as the head and shoulders of the seals being completely on dry land

Since the study aimed to test how “*phocid*” seals respond to sound, the data for both the 6 grey seals and 2 harbour seals were pooled. This was based on the assumption that at least with respect to hearing sensitivity both species are similar. A behavioural audiogram is currently not available for grey seals. However, in spite of differences in body size many *phocid* seal species do seem to have similar underwater audiograms (compare Terhune, 1988; Terhune & Ronlad, 1972; Terhune & Ronald, 1975). For all considerations with respect to sensation level calculations I used a composite audiogram based on Kastak & Schusterman (1998), Terhune (1988) and Mohl (1968) (see appendix 1). I did not use data from highly invasive electro-physiological measurements (cortical response measurements by Ridgway & Joyce, 1975) on grey seals since it is not known how these measurement relate to behavioural audiograms (e.g. with respect to absolute sensitivity). Statistical tests were calculated in SYSTAT 11 with the exception of the General Linear Models which were calculated in JMP 4 (SAS). In one case part of the data for a response variable was tested twice in different models. In that case an adjusted p-value is mentioned. However, given that the Boneferroni adjustment has come under strong criticism (Nakagawa, 2004) this should be looked at with caution.

Results

Index of aversiveness: Short-term efficiency and habituation

The median index of aversiveness was used to summarize the most extreme aversive responses for all eight animals in each of their first playback sessions. While seals never hauled out in response to any sound they showed medium aversive responses up to level three (turn away, flight & prevention of fish catch). As can be seen in figure 1 aversive responses in the first playback session were in the same order of magnitude for all sounds. There was a significant difference in the index of aversivness between the four treatments (no sound, control sounds, new sounds, seal scarer) when the median was calculated over all sound types used in each treatment for all eight individuals (Kruskal-Wallis $H=9.383$, $p=0.025$, $df=3$). Aversive responses declined rapidly during the first playback session and median responses were zero for all sounds in the second playback session and in all following sessions (fig 2b). Although sound exposure did elicit an occasional “head turn away response” in some animals in the second playback session there was no significant difference in the median index of aversiveness between the four treatments (Kruskal-Wallis $H=5.907$, $p=0.116$, $df=3$) indicating that sound exposure had lost its effect. Since responses of seals did not differ by sound type and all sound types were presented in a different pseudo-randomised order to each individual, it is likely that playback order was the most important factor influencing response amplitude.

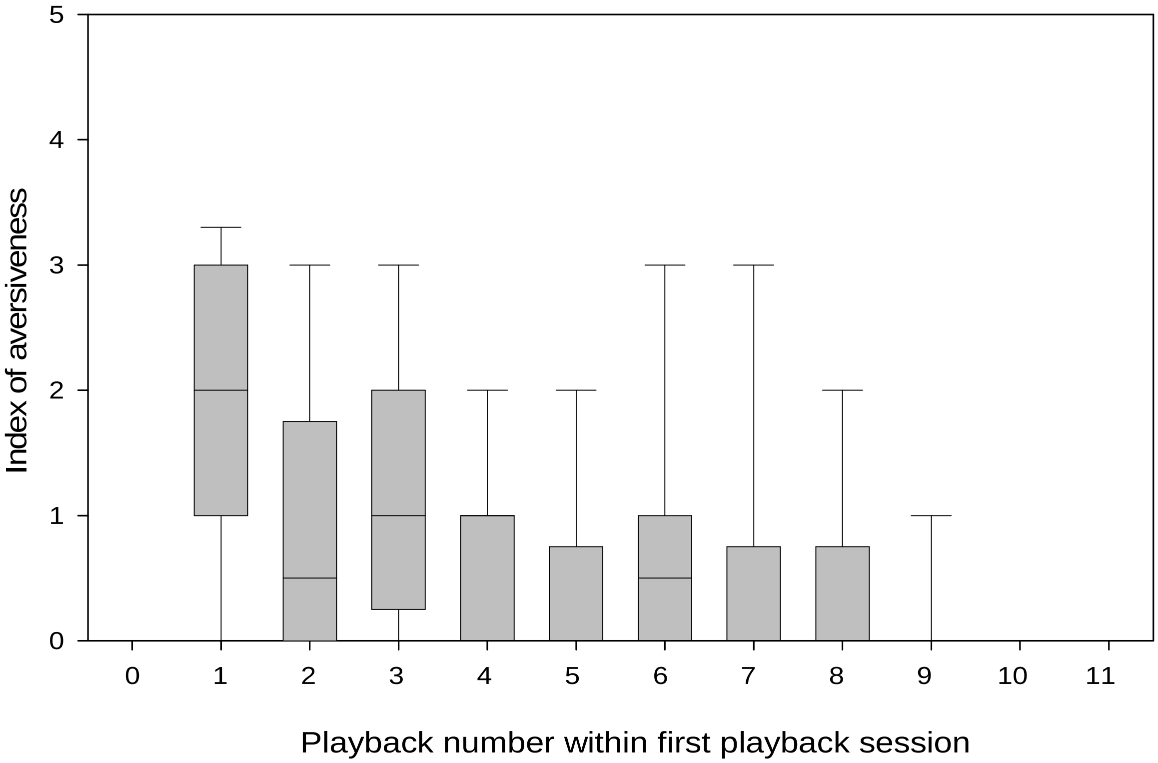


Figure 3: Habituation of responses within the first session independent of sound type. Data are median, interquartile ranges and 90% margins for all seals. Since each seal had a different sequence in which sounds were presented, data are listed by playback position in the first session. The data shows the response decline to zero within the first playback session

Figure 3 shows the median responses for the eight seals ordered by playback number within the first playback session. There was a strong decline of the responses over first 3 to 4 playbacks with median responses reaching zero in all trials following the 7th playback. A highly significant difference in the median seal responses tested by playback number indicates that playback order was in fact a crucial factor (Kruskal-Wallis, H=25.126 p=0.005, df=10). Furthermore, a Spearman rank correlation test revealed that there was a highly significant negative correlation between the median response score and playback number indicating fast habituation (t=-6.36, p=0.00013; see also fig 3). Interestingly, playback number within the first session did in fact explain 82% of the overall variation in the index of aversiveness. This clearly shows that the response magnitude to a certain sound primarily depends on when it was played to a seal within the first playback session. As can be seen in figure 3, a sound had the highest likelihood to elicit a strong response in the first playback session if it was among the first 2-5 sounds a seal had ever heard in the test pool. However, in the second and all following playback session all responses were zero and playback order did therefore not matter anymore.

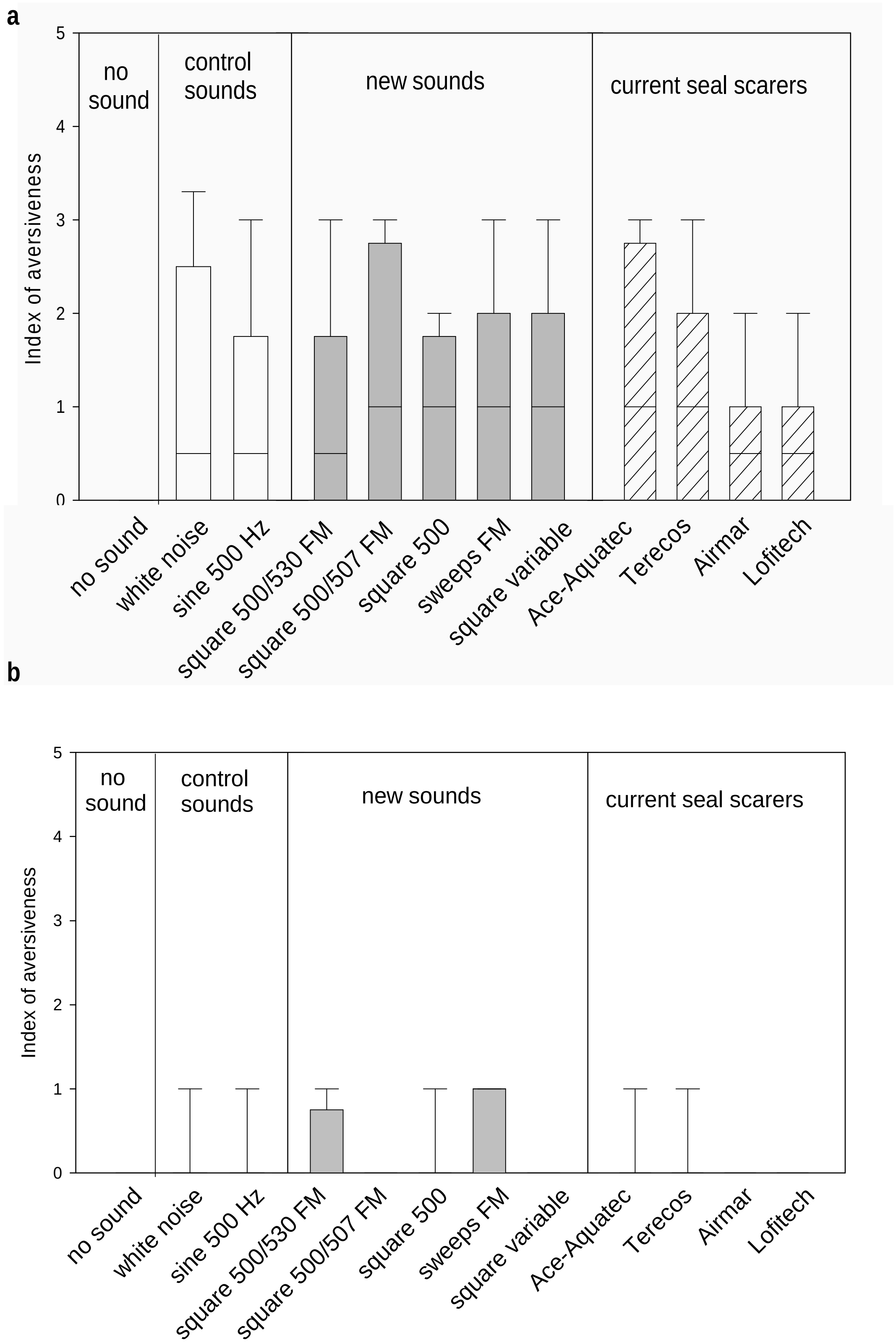


Figure 2: Responses of the eight seals to the following treatments: no sound, control sounds, new sounds and seal scarer sounds in a) the first playback session and b) the second playback session (both trials included food motivation). The data are median, interquartile ranges and 90% margins for all seals. Note the habituated responses in the playback second session.

Longer-term responses: Time spent close to feeding station and dive time

In contrast to the quick habituation process with respect to the most obvious aversive responses (index of aversiveness), sound exposure seemed to maintain some effect on the continuous response variables “dive time “ and “time spent close to feeding station”. Graphical evidence shows that exposure to any of the three sound treatments (control sound, seal scarer or new sound) reduced the time an animal spent close to the feeding station and caused a reduction of dive time over the course of several playback sessions (see fig 4.). In order to elucidate potential factors that might influence swimming and diving behaviour in the pool general linear models were calculated for the mentioned response variables over all sessions that involved food presentation (see figure 4, tables 1 & 2). The model included playback session number, individual identity, treatment and all three interaction terms as variables. The model for “time spent close to feeding station” was highly significant ($F_{64,124}=8.14$, $p<0.0001$) explaining 71% of the variance in the data. The results of the model and the biological meaning of the variable are summarized in table 1. Individual identity was the most important explanatory variable in terms of time spent close to the feeding station, followed by treatment (effect of sound exposure) and to a lesser degree playback session number. The interaction term for playback session number and individual identity was also marginally significant while all other interaction terms did not contribute significantly to the model. Generally, seals reduced the time spent close to the feeding station slightly in later playback sessions in all four treatments. However, the interaction term “treatment X playback session” was not significant showing that the effect of sound exposure on behaviour did not change over time (no clear habituation). The parameter estimates from the model revealed that the effect of treatment was due to the difference between the no sound control and sound exposure while there was no significant difference between the sound types. The model for dive times explained 85% of the variance and was highly significant (GLM, $F_{64,124}=12.22$, $p<0.0001$). Similar to the model for “time spent close to the feeding station” the most important explanatory variable was individual identity. However, in contrast to the previous model the second most important factor was playback session number followed by treatment. This reflects the observation that seals decreased dive time in later playback sessions in all four treatments. The interaction terms “individual x playback number” and “treatment x individual” were also significant but contributed little to the model. However, treatment was not significant

Variable (sample size)	**Biological meaning of variable**	**Time close to feeding station**		**Dive time**	
		F	**p**	**F**	**p**
Treatment	Effect of sound exposure and sound type on behaviour	4.4754	**0.0051**	5.514	**0.002**
Playback session no	Behavioural changes over time	2.8137	**0.0421**	10.195	**<.0001**
Individual	Individuals behave differently but do not necessarily respond differently to sound	22.689	**<.0001**	30.0449	**<.0001**
Treatment x Individual	Individuals respond differently to sound exposure or sound type	1.2734	0.2059	1.8766	**0.0289**
Treatment x play-back session no	Habituation to sound exposure or sound type	0.661	0.7427	0.98	0.4652
Individual*play-back session no	Individuals change behaviour differently over time	1.9562	**0.0126**	2.1084	**0.0121**

Table 1: General linear model for the four food trials for the response variables “time close to feeding station” and “dive time “

Variable	**Biological meaning**	**Playback session:** **3 (food) vs. 4 (no food)**				**Playback session:** **5 (food) vs.6 (no food)**			
		Time close		**Dive time**		**Time close**		**Dive time**	
		F	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Food presentation	Level of food motivation	1.30	0.2592	0.072	0.79	8.61	**0.005**	7.18	**0.010**
Individual	Individuals behave differently	29.42	**<.0001**	28.69	**<.0001**	15.07	**<.0001**	26.58	**<.0001**
Treatment	Effect of sound exposure	3.33	**0.020**	3.34	**0.0262**	0.1762	0.912	1.601	0.2002

Table 2: Comparison of consecutive food and no food trials for the response variables “time close” and “dive time” using multifactorial ANOVAs. Significant difference for a variable are marked in bold.

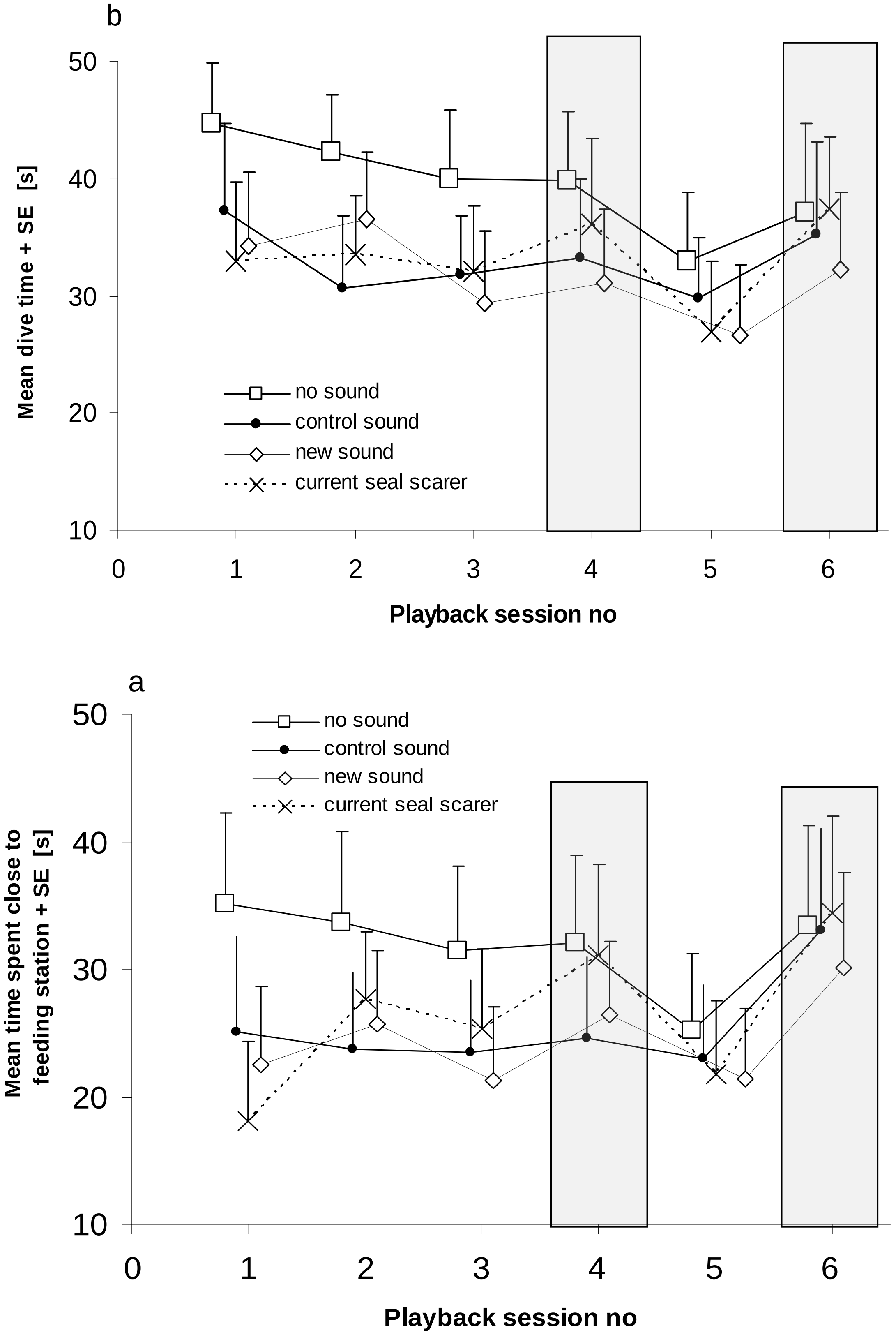


Figure 4: Dive time (a) and time spent close to the feeding station (b) in response to playback of sound falling into the categories: New, aversive sounds, currents seal scarers and control sounds. Data points are mean values plus standard error. In sessions with shaded grey bars no food was presented to the animal.

meaning that sound exposure did not influence behaviour significantly in the last two trials. None of the p-values would be affected by Bonferroni adjustment for multiple testng on the same data set

In order to test for differences in behaviour between consecutive playback sessions with and without food presentation I used multi-factorial ANOVAs including individual ID, treatment and food presentation schedule (food vs. no food) as covariates. The comparison model for playback sessions 3 (no food) and 4 (food) was significant for both response variables, time spent close to feeding station ($F_{11,63}$=19.748, p<0.0001, r^2=0.77) and dive time ($F_{11,63}$= 19.175, p<0.0001, r^2=0.76). The model showed that there was strong inter-individual variability in dive times and time spent close the food source as well as an effect of treatment. However, no effect of the food presentation regime was found (see table 2). If p values (table 2) were adjusted for multiple testing (Bonferroni) treatment would lose its effect on dive time but the effects of all other variables would be unaffected. The comparison models for playback session 5 and 6 were also significant for both response variables (dive time: $F_{11, 63}$=10.42 p<0.0001, r^2=0.62; time close $F_{11, 63}$=18.00 p<0.0001, r^2=0.75). In contrast to the previous models, food presentation regime (food versus no food) had an influence on “dive time” and “time spent close” in the models comparing behaviour in 5th (food) and 6th session (no food). This means that seals dived longer and spent more time close to the feeding station when no food was presented (see fig 4). However, again individuals showed strong differences in their general diving and swimming behaviour. Interestingly, treatment was not significant meaning that sound exposure did not influence behaviour significantly in these last two trials. None of the p-values would be affected by Bonferroni adjustment for testing the same data set twice.

Discussion of first experiment

This first experiment gives a good impression on how seals react to artificial sounds when near a known food source. It appears that while they show an avoidance response initially, they habituate very quickly and only show longer term reactions in parameters that do not affect their foraging success. These sounds might therefore not be able to permanently keep seals from a known food source. The fact that all seals habituated within the first playback session during which 11 different acoustic stimuli were presented indicates that variable stimulus design is unlikely to be effective in preventing habituation (as some manufacturers have suggested; e.g. oral communication by Terecos ltd.). The novel stimuli caused the same type of aversive responses as stimuli used in commercially available ADDs. Since the way I designed these stimuli predicts that they would not have the same detrimental effects on odontocetes that have been described for commercial ADDs it may still be beneficial to use them in ADDs. However, since none of the sounds were effective in deterring seals in experiment 1 such an approach is questionable. In addition the data show that different levels of food motivation effect behavioural responses to sound as indicated by the fact that seals were willing to tolerate the sound exposure more readily (stay longer underwater close to the feeding station) when no food reward was given. Possible reasons of the more complex behaviour observed in the continuous response variables (time close, dive time) will be discussed together with the results from experiment 2 in the general discussion with respect to habituation theory, possible conditioning processes and motivational state. In general the data shows that in the tested experimental setup strong food motivation (presentation of food) was most likely an important factor that led to to fast habituation to all tested playback sounds.

Experiment 2: Responses of seals in the wild without food motivation

Methods

8 of the 11 stimuli from the previous experiment were tested in the wild near a haul-out site for grey seals. The field site was at Abertay Sands close to Tentsmuir Forest, Fife (Scotland/UK). The area consisted of sandbars some of which extended several kilometers offshore from the mouth of the Tay Estuary. Grey seals haul out in the area on four haulout sites, one of which is close to the main foreshore, two are located on sandbars close to the foreshore and one is further offshore on the outer sandbars. The overall number of grey seals using the haulout sites in the Abertay Sands area in summer is about 1500 (see Tentsmuir NNR: Reserve Management Proposals, http://www.snh.org.uk). Haulout sizes during the experiment ranged from approximately 20 to 200 animals. Playbacks were carried out at all four haulout sites and playback sounds were attributed equally to the sites. The haulout site was approached from sea with a RHIB. After a step-wise “stop and go” approach the boat was anchored between 80 m and 250 m from the haulout. The playback source was deployed at a depth of approximately 1.5m at the stern of boat. The playback

equipment was the same as that used in the captive experiment. Observations were carried out starting 5 min prior to playback (pre), 5 min during the playback (sound) and for 5 min following the playback (post). A 15 min recovery period separated each of these 15 min observation blocks. Each 5 min playback trial consisted of just one sound type. Not more than 5 playbacks (5 x15 min observations periods each followed by a 15 min recovery period) were carried out on one day. A playback was excluded from analysis if no animals were seen closer than 50m during the pre-observation period. This was done because in that case no animals could have been deterred from an area where the sound was most likely to elicit a response. Also, using such playbacks would have dramatically reduced the statistical power of detecting differences between pre and sound exposure observation periods and it would have not been possible to say whether a deterrence effect existed at distances closer than 50 m. Also, “no sound” observation periods of 15 minutes were carried out as a control. A “no sound control” involved the normal 15min observation period with the observer behaving the same way and the equipment being deployed in the water, however, no sound was played during the 5min between the pre and post observation period. This was done on 16 days but two were removed due to the criteria for the minimum number of animals having to be present closer than 50m (n=14).

The sequence in which sound types were used was pseudo-randomised. No sound type was tested in more than one playback on the same day. The order in which different sound types were presented on a playback day was pseudo-randomised. Since 8 different stimuli were tested not all stimuli were tested each day, however, sound stimuli were distributed evenly with respect playback days, intervals between playback and haulout sites. Sounds were played at a source level of 172 dB re 1 µPa (rms) @ 1 m for 10 s followed by 10 s of silence for a 5 min period (duty cycle was therefore 50%).The playback stimuli were the same as in experiment 1, however, only the two new sounds that were most efficient playback session 1 in experiment 1 were tested (Sweeps FM. Square 500/530 FM). The two control sounds and the new sounds were all tested 10 times on separate days within a period of several months. Current seal scarer sounds could only be tested 6 times due to time constraints. Surface positions of seals were measured continuously relative to the playback boat using a laser range finder (Bushnell Yardage Pro 1000) and a handheld compass. Seals surfacing at a distance of more than 100m from the source were not included in the observations and analysis. This was done because the detection probability dropped rapidly at distances further away from the sound source. The source level was therefore chosen to result in a gradient of received levels that would most likely only cause an avoidance response at distances much smaller than the whole observation area (see introduction for expected discomfort threshold in seals). I conducted playbacks on 18 separate days in 2006 and 2007. The data were analysed by conducting repeated measures ANOVAS comparing the number of seals between pre, sound and post observation periods in distance bins of 20 m. Deterrence ranges were defined as the outer edge of the distance bin furthest away from the sound source in which the number of seals was significantly lower during sound exposure.

The measurements of the sound field around the haulout site were done from a small inflatable boat while the sound source was operated in the usual way from the main RHIB. The inflatable was anchored during recordings and distances were measured with a laser range finder by a person on the main RHIB. Sound field measurements were conducted at just one haulout site on the outer sandbars in the mouth of the river Tay. This was the haulout site where more than 75% of the playbacks were carried out. All playback sounds were played consecutively and measured received levels were averaged over all 8 sounds. Received levels were measured along two depth profiles, one parallel to shore and a second one from the boat to the shore. Water depth along the profiles was between 3.5 m and 5 m for the first and ranged between 4.5 m and 1 m for the second profile. The measured received levels along both profiles were also used to determine avoidance thresholds (received level at the edge of the deterrence range).

Results

Distribution of animals by distance bin

The distribution of animals in the 5 distance bins did not differ significantly between the three 5 min observation periods for the no sound control (fig.5). This shows that the experimental setup and the behaviour of the observer did not result in changes of seal distribution. Figure 5 also shows that while the detection rates of seals were

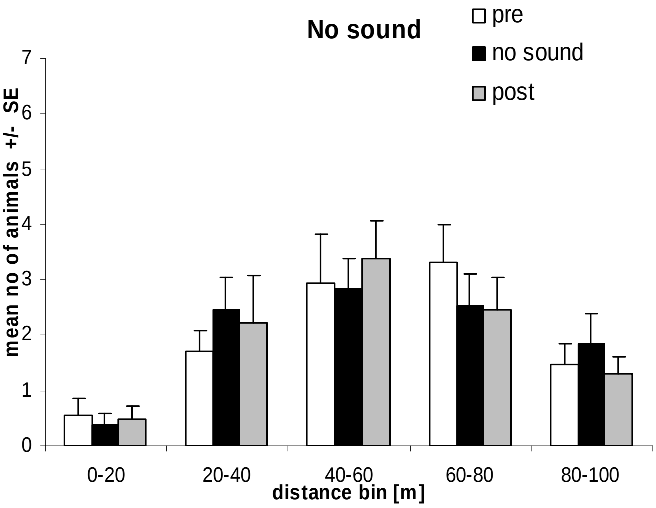


Figure 5: Number of seals surfacing in different distance bins from the speaker (and nearby boat) in the wild in control trials when no sound was played (n=14 observation periods).

similar at distances between 40m and 80 m the likelyhood of sighting seals at disances 80-100m was lower. In contrast to the distribution of seals during the no sound control there was a significant decrease in the number of animals in at least one of the distance bins for all tested sound types (fig 6, repeated measures ANOVAs all p>0.05). The only exception was the sound of the Terecos seal scarer which did not cause a significant reduction of seal numbers in any of the distance bins. It should however be noted that all seal scarer sounds were only played 6 times resulting in a lower statistical power of the tests. Deterrence ranges for the new sounds were 60m (Sweeps FM) and 80m (Square 500/530 FM) respectively. Deterrence ranges for the control sounds sine 500Hz and white noise were 40m and 60m respectively. The seal scarer sounds of the manufacturers Ace-Aquatec and Lofitech yielded a deterrence range of 60m while the deterrence range for the Airmar device was 40m. No deterrence range was found for the sound of the Terecos device.

Deterrence effects within 60m of the sound source

A comparison of the deterrence effects of the different sound types in an area closer than 60m from the sound source revealed that there was a significant difference between the 8 tested sounds (Kruskal-Wallis, H=15.424, p=0.031, figure 7). This might be mainly due to the lack of a clear deterrence effect in case of the Terecos seal scarer. However, figure 7 also shows that some sound types were slightly more effective in clearing the area of seals (e.g Sweeps FM, Lofitech & Ace-Aquatec).

Larger scale movement patterns and longevity of deterrence effects

In order to test how far animals moved away and if they left the overall observation area the number of animals closer than 100m was compared between the pre observation period and sound exposure. A significant drop in seal numbers closer than 100m was only found for one of the tested sounds, one of the supposedly more unpleasant new sounds: Square 500/530 (Friedman test, p<0.002). However, for two tested sounds a significant difference between pre- and post-observation period was found. These two sounds both belonged to the category “new sounds”: Square 500/530 FM (Friedmann test, p=0.02) and Sweeps FM (Friedmann test, p=0.02). This means that in both cases seal numbers were lower5min after sound exposure

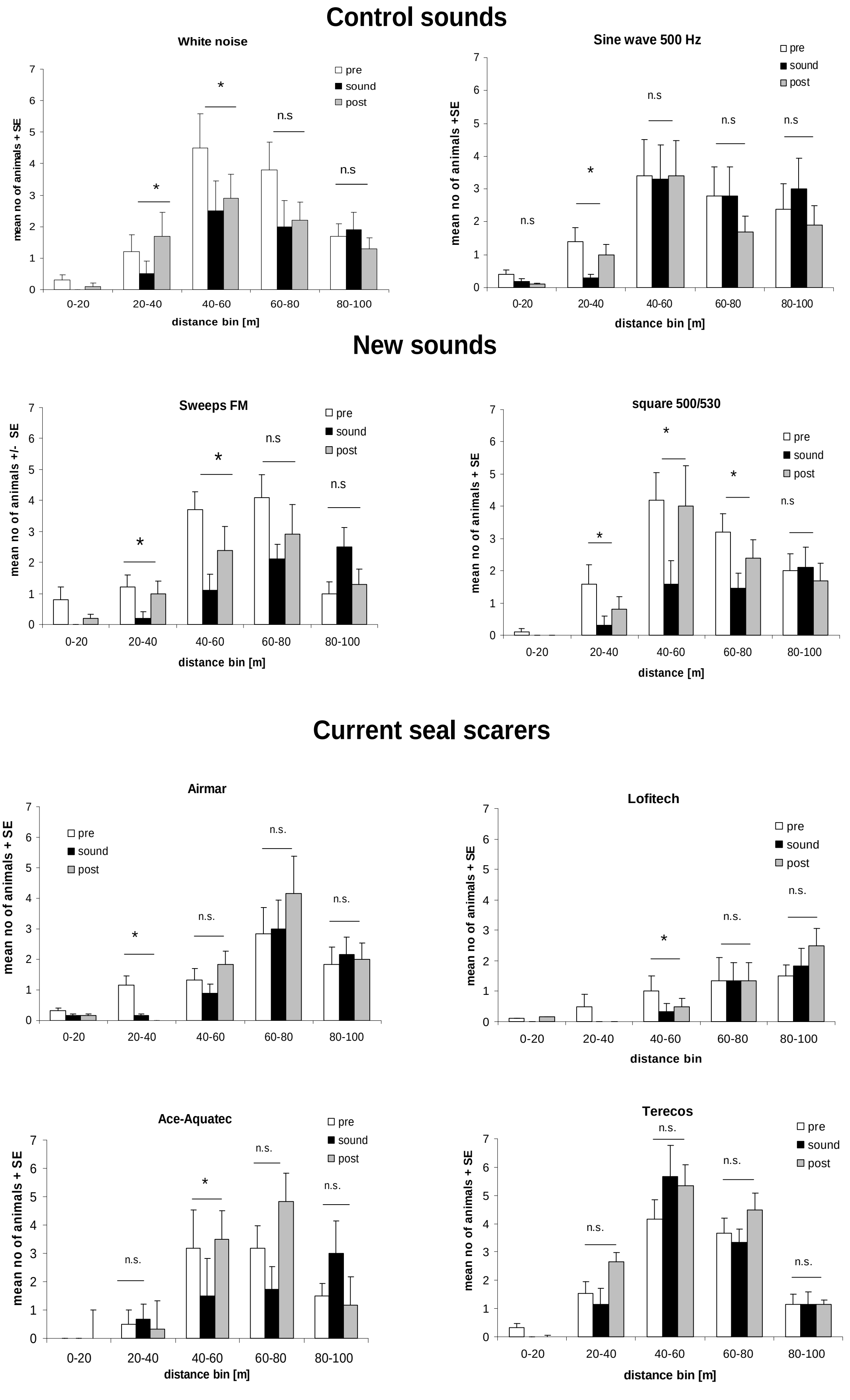


Figure 6: Reactions to no sound, control sounds, novel sounds and seal scarer sounds in the wild without food motivation nearby boat.

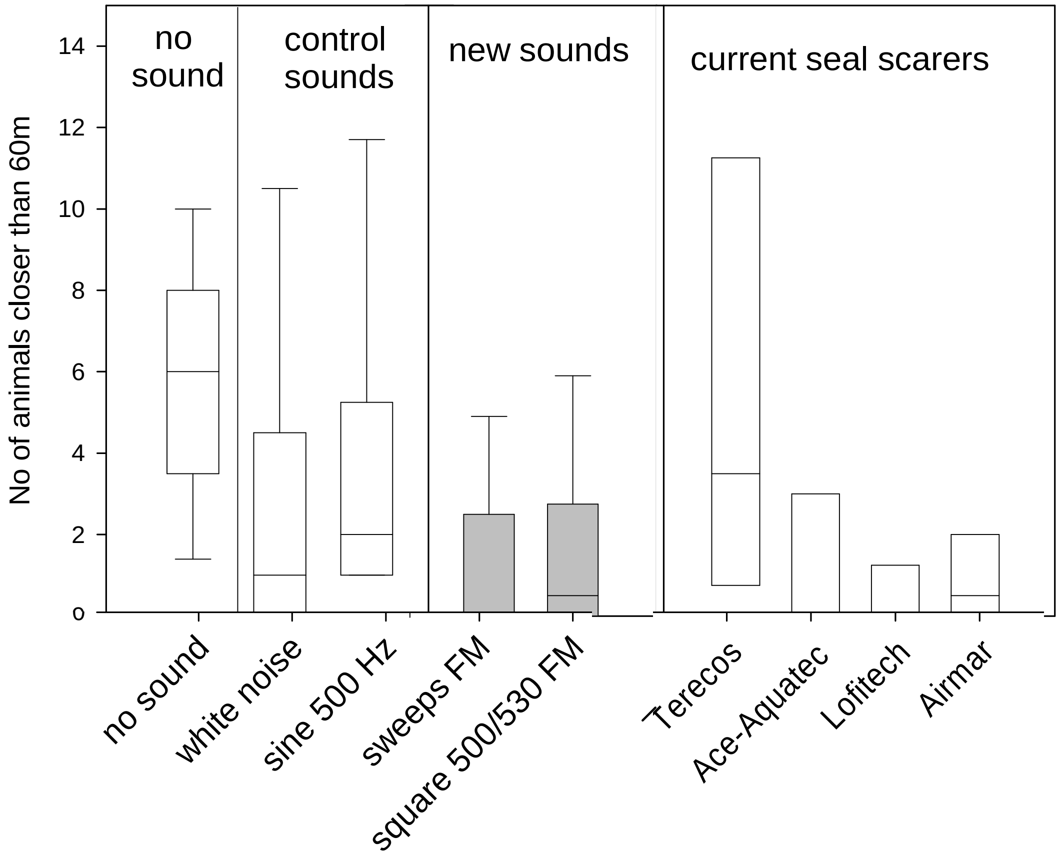


Figure 7: Deterrence effects of the 8 different sound types within an area of up to 60m from the sound source. The data show median, interquartile ranges and 90% margins for 10 (control & new sounds) and 6 playbacks (seal scarer).

compared to the 5 min before sound exposure started. In case of the Square 500/530 FM even if the p-value was Bonferoni adjusted for multiple testing on the same data the result would still be significant (p=0.04). Given that two sound types caused a deterrence effect that extended to at least 5min post sound exposure over the whole observation area it is in theory possible that not all animals returned during the 15 min recovery periods. This could have potentially biased the following playback. However, a comparison of all 5 min pre-sound exposure observation periods for each playback day reveals that the mean number of animals within the observation area did not differ between consecutive playbacks meaning that no drop of seal number occurred over the course of a playback day (ANOVA $F_{4,\,63}$=1.44, p=0.23). This shows that while not all animals returned during the 5 min after sound exposure ceased (post periods) the 15min recovery time was sufficient for all animals to return to the observation area. Alternatively, it is possible that the area was filled up with new arrivals during the post-playback phase.

Habituation within one playback day

In order to test habituation effects to repeated exposures within one playback day the number of animals closer than 60m from the playback source was counted for all playback sessions. This was based on the assumption that if there had been significantly more animals present during the 5min of sound exposure later in the day this would be most likely due to habituation. In contrast if no differences were found this would not preclude habituation since there might have been a turnover in animals. Although the data scattered towards higher values (see fig 7) meaning that on some playback days more animals were present in later playbacks there was no significant difference in the number of animals between any of the playback sessions (Kruskal-Wallis $H_{4,17}=8.820$, $p=0.116$)

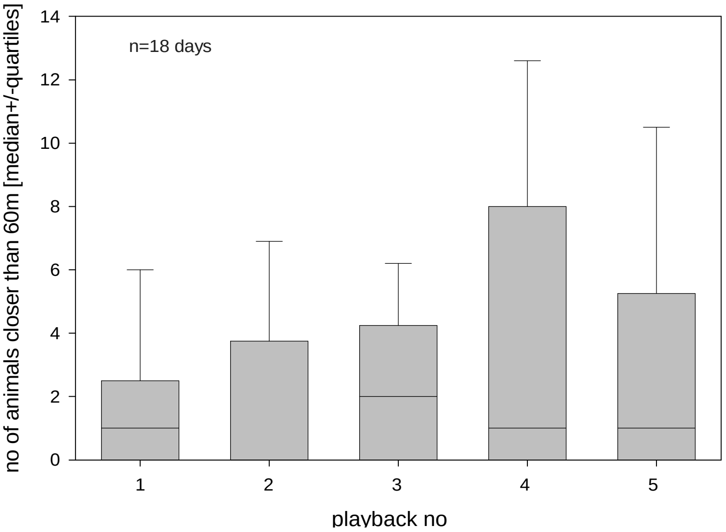
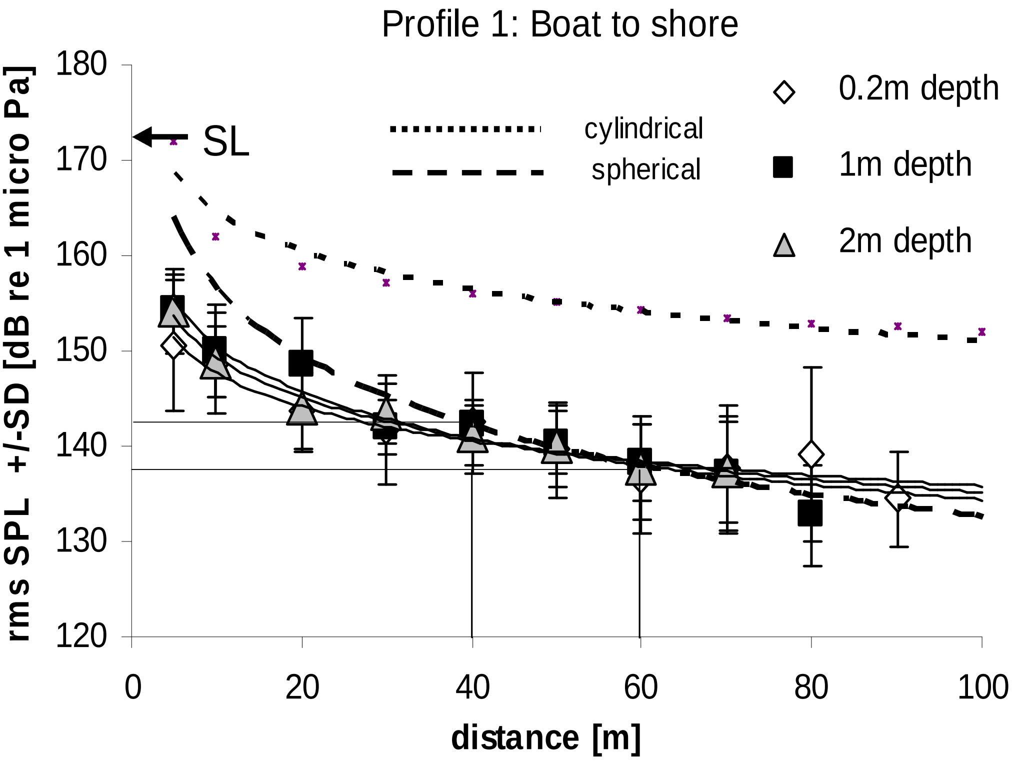


Figure 7:Median number of seals closer than 60m during sound exposure for the maximum of 5 playback that were carried out on each playback day. The graph shows that seal number did not increase over the course of a day.

Sound field

The data from the sound field measurements are presented in figure 8. In the profile measured from the sound source towards the haulout site on the shore, received levels at different depths were rather similar. Transmission loss was higher than would be expected by either cylindrical or spherical spreading in the first 20m but then tailed off as predicted from spherical spreading. In the



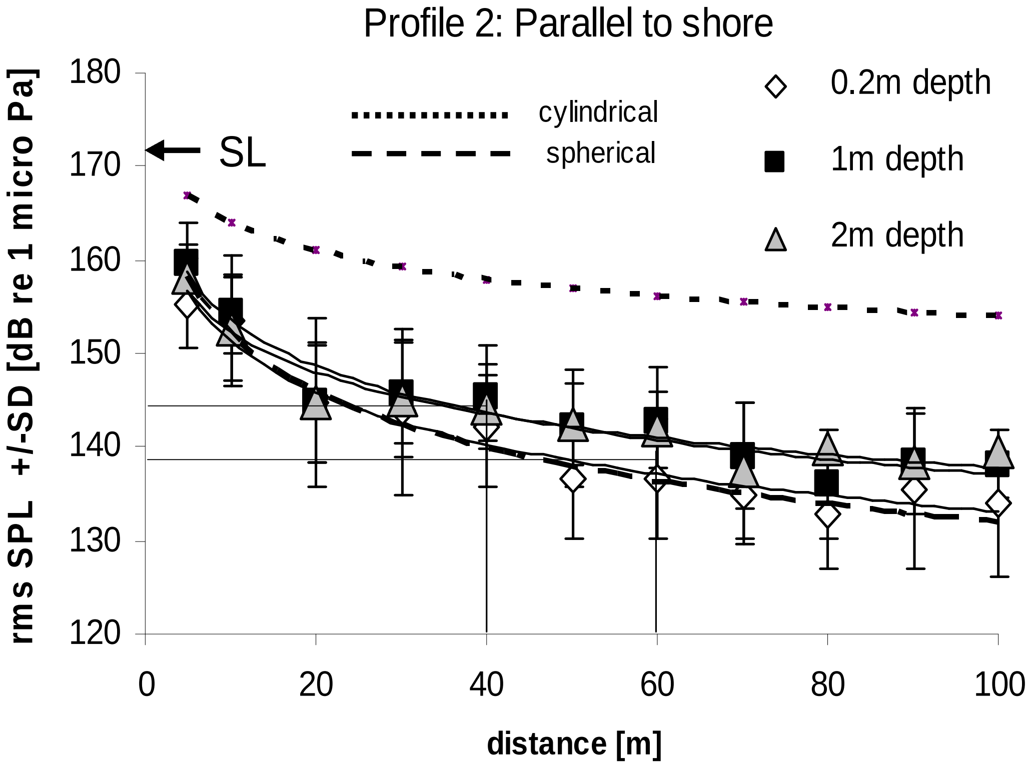


Figure 8: Measured received levels along the two profiles from the sound source to the foreshore and parallel to the shore at 0.2m, 1m and 2m depth. The expected received level based on cylindrical and spherical spreading and the measured source level (SL) are depicted by dashed lines. The black lines show the received level at the edge of the most common deterrence ranges (area within which significantly less seals were sighted).

profile parallel to the shore, transmission loss was closer to cylindrical than spherical spreading. Received levels right underneath the water surface (0.2m) tended to be lower compared to deeper measurements. In spite of the complex habitat transmission loss under representative conditions was close to predictions obtained from spherical spreading. The arrows in fig 9 indicate received levels at the edge of the distance bins where deterrence occurred.

General discussion

Evaluation of the field data: Do changes in seal abundance in the field trials reflect individual movement patterns?

A potential problem with the field trials is that changes in the number of animals in the respective distance bins might not reflect individual movement behaviour. However, in the light of the sound field measurements (see fig. 8) the most efficient behaviour for a seal to remove itself from an area of high received levels under test conditions in experiment 2 would be a horizontal movement response directed away from the sound source. Also, some well marked individuals that were recognisable by their pelage pattern on the head or the shape of their nose (adult males) could be identified over several consecutive sightings. In many cases those individuals were first seen close to the boat, then surfaced at further distances during sound exposure looking towards the playback boat. On many occasions animals were seen to return to the boat after sound exposure had ceased. In addition, animals surfacing at distances of 15-40m from the boat with their head half-submerged exhibited sudden dive responses (“crash dives”) at the start of the sound exposure. This was usually followed by a 1-2 minute period with few surfacings but then a similar number of animals re-emerged at distances of 60-150m from the playback boat. Additionally, as shown in figure 7 there is no evidence that the number of seals changed between the three 5min observation periods in any of the distance bins as a result of factors other than sound exposure (e.g. observer behaviour).In two cases a seal could be observed through the water surface at distances of 7-10m when the sound was switched on. In both cases the seal turned away and moved away from the sound source. Finally, a pilot trial with a high-frequency scanning sonar (Tritech Sea King) was carried out (15min observation periods) and showed that while many echoes were difficult to classify 60-70% of the clear detections with the sonar also had corresponding visual surface detections in a similar area.

There are also potential problems involved in using an analysis in 20m distance bins. For instance the area covered by each distance bin is not identical meaning that absolute numbers of animals do not reflect density of seals. It should also be noted that the lower detection rate of seals at distance of more than 80m might have reduced the likelyhood of obtaining a significant result using the repeated measures ANOVA. However, the fact that generally more animal were sighted in the distance bin from 80m-100m during sound exposure would indicate that deterrence ranges were limited to closer distances and some animals moved away into the 80m-100m distance during sound exposure.

What factors other than sound type influence a seal’s response? Food motivation and conditioning processes

In spite of the lack of experimental evidence several studies have pointed towards an influence of motivation on the behavioural responses to noise in marine mammals. For instance, pinnipeds habituated to acoustic deterrent devices (Mate & Harvey, 1987) while odontocetes exhibited aversive responses for years with no signs of habituation (Morton & Symonds, 2002; Morton, 2000). Early models of motivation claimed that a behaviour specific “energy” accumulates until it reaches a certain level and therefore elicits a certain behavioural response (Lorenz, 1950). Although some recent authors have argued for their usefulness (Hogan, 1997) energy models have generally been criticised (Hinde, 1960). Alternatives include the concept of “motivational state” which can be interpreted as a multivariate vector space in which several physiological and behavioural factors interact (Sibly, 1974). In the presented experiments seal responses to sound were tested under three different conditions with respect to food availability and motivation: 1.) Foraging behaviour on a profitable food source with some food motivation being present since the animal had not been fed for about 12 hours. 2.) Behaviour around an empty (“exploited”) food source which the animal knows to be profitable from previous trials (experiment 2, no food trials). 3.) Behaviour of seals around a haul-out site where animals were presumably not food-motivated since the area around the loudspeaker is not known to be used for foraging (experiment 3). Food availability and motivational state might have influenced behaviour in this study in several ways. Seals did not respond differently to different sound types in the experiments involving food motivation while unmotivated animals in the wild differentiated between sound types. This observation may have to do with the fact that food motivation was overriding any possible dislike of sounds in the captive experiments causing the animal to tolerate sound exposure and stay close to the feeding station. The observed decrease in time spent close to the feeding station and dive time could be due to a decrease in motivation. However, trying to explain the seals' behaviour by motivational state may be overly simplistic. Some of the responses could also be interpreted as the result of conditioning processes which might provide a better explanatory basis than motivation theory. While the food presentation can be interpreted as an unconditioned stimulus (UCS) the presence of the edge of the cup (that contains the fish) and the playback of the sound during foraging can be interpreted as conditioned stimuli (CS). The animal positioning itself in front of the feeding station even without any food presentation can therefore be regarded as a conditioned response (CR) in the Pavlovian sense (Pavlov, 1927). This would mean that the behaviour of ignoring the sound has successfully been put on cue and the seal was exhibiting a stereotyped behaviour in response to the appearance of the cup and the playback of the sound. However, this would require stimulus generalisation within the acoustic modality since the presented sound types differed substantially. Acoustic CS generalisation depends on the level of discrimination training an animal has received but generalisation might in fact be low for naïve animals (Jenkins & Harrison, 1960). The observed behaviour can also be interpreted as having an operant component since the animal learns to manipulate the cup and therefore to obtain a food reward. In conditioning procedures intermittent reinforcement schedules have been shown to result in stronger responses and higher resistance to extinction compared to continuous reinforcement (Skinner, 1933). The response in payback session 6 could therefore also be a result of the lack of a food reward in playback session 4. It seems important to note that although the observed conditioning processes originate from the experimental setup many observed cases in which wild animals exploit profitable artificial food sources are likely to be based on the same processes (see Jefferson & Curry, 1996 for examples of the so called "dinner bell effect" where habituated seals are attracted to an acoustic deterrent device).

Habituation processes

Groves & Thompson (1970) developed a “dual-process” theory of habituation suggesting that “…the strength of the behavioural response elicited by a repeated stimulus is the net outcome of the two independent processes of habituation and sensitisation (p. 442)”. The theory makes several predictions about differences between habituation and sensitisation processes which are consistent with reviewed empirical data as well as data collected by the authors themselves. In addition the dual process theory gained strong support from experiments on habituation of acoustically elicited reflexes (Pilz & Schnitzler, 1996). In experiment 1 the most aversive responses as measured by the index of aversiveness habituated within the first playback session. However, the impact of sound exposure remained significant in more subtle response variables. Given that playback session number was significant in the model for all food trials one can conclude that seals decreased their dive time and the time spent close to the feeding station during both control and sound exposure towards the end of the experiment. The effect of sound exposure on the seal’s behaviour did not change over time (interaction term playback session number x treatment was not significant). Therefore, in spite of the fact the animals did not sensitise to the sound exposure per se the decrease in dive time and “time close” can be interpreted as a weak sensitisation process with respect to the movement behaviour of the seal in the pool. Given that sounds were played in 92 % of the 1 min playback observation periods during which seals obtained food, sound exposure as part of the experimental setup is likely to have played a role in this process. According to the dual process theory this would mean that the overall observed behaviour of the seals can be interpreted as the net outcome of two processes: 1.) a rapid habituation process with respect to the flight responses and the recovery of prevented foraging behaviour 2.) a weak sensitisation process with respect to place preference and diving behaviour in the pool.

There is no clear evidence for habituation in experiment 2 with wild seals in a context where no food motivation was involved. Although the scatter of the data towards higher values in later playback sessions might suggest that slightly more animals were close to the sound source this difference was not significant. The most likely explanation for the lack of habituation might be that there was a turnover in animals close to the boat e.g. some animals might just have passed through the area while approaching the haulout site. Alternatively, animals could have been displaced by the sound exposure and replaced by new arrivals. However, since in some cases well-marked individuals were seen over the course of several playbacks it is also possible that seals did simply not habituate e.g. because the cost for temporarily leaving the area was rather low (in the captive experiment animal could have left the pool to remove themselves from the sound exposure but they did not). This would be consistent with the results from captive experiments on marine mammals that failed to find rapid habituation in response to a variety of different acoustic stimuli in contexts where motivation to stay close to the sound source was low (Kastelein et al., 2006; Kastelein et al., 2005; Kastelein et al., 2006b). There is also indirect evidence from a variety of field and lab experiments pointing towards the importance of food motivation in facilitating quick habituation processes (as it was found in this study).

Wild odontocetes do not seem to habituate to acoustic deterrent devices in areas where these species do not forage on farmed fish (Morton & Symonds, 2002; Morton, 2000). In contrast food motivated seals habituated to these devices at least under certain circumstances (Mate & Harvey, 1987) and a total lack of any deterrence effect was found in areas where ADDs are frequently used on fish farms (Jacobs & Terhune, 2002; Mate & Harvey, 1987). Similarly, captive sea lions habituated relatively quickly to artificial sound at sound pressure levels of 165 dB re 1 µPa in a context where foraging was simulated (Akamatsu et al., 1996). However, it should be mentioned that there is also one study on captive harbour porpoise that showed that habituation can occur even though the animals were not foraging and costs to avoid the area close to the sound source were probably rather low (Teilmann et al., 2006).

The data from experiment 1 clearly showed that variable stimulus design was not successful in delaying habituation of the more extreme avoidance responses (e.g. flight) and habituation took place within the first playback session. This is in contrast to statements given by one manufacturer of ADDs that claim to have developed a highly effective way of preventing habituation by using complex and variable stimuli (oral communication by Terecos). However, according to Groves & Thomson habituation theory stimulus generalisation will depend on whether common features in the “stimulus-response pathway” are shared between stimuli. Since all stimuli used in this study are perceived through the auditory pathway and sensation levels differed by not more than 15 dB stimulus generalisation is in fact well in line with the dual-process theory.

The results show that the sounds of current ADDs as well the new sounds tested in this study were unable to deter a predator from a food source at the tested received levels for an extended amount of time. According to habituation theory the extent and speed of habituation is inversely related to stimulus frequency (duty cycle) and intensity (Groves & Thompson, 1970). This means that it is possible that habituation would have been delayed if higher source levels had been used. However, the relatively high duty cycle would still have contributed to habituation at the chosen received level. More importantly the chosen received level (146-147 dB re 1µPa) is still within the range within which manufacturers of high duty cycle and high intensity seal scarers claim their devices to be efficient. For example in case of a Lofitech seal scarer with an output 189 dB re 1µPa @ 1m the received level used in this study would be reached at 140m distance (assuming spherical spreading without absorption). The manufacturer however claims a deterrence range of up to 300m (information from the manufacturer’s website http://www.lofitech.no). The test conditions in experiment 1 can therefore be considered realistic with respect to received level in the pool meaning that the data can be used to make predictions about long-term effectiveness of ADDs. Furthermore, the chosen received level in experiment 1 was higher than the received levels at the edge of the deterrence range for most sounds in the field trials which shows that received levels were sufficient to deter unmotivated animals. With respect to current seal scarers one should however note that one manufacturer (Ace-Aquatec) sells a motion trigger for its device which can potentially reduce the duty cycle dramatically. Ace-Aquatec also claims a realistic deterrence range of 50-80m for their 194 dB re µPa device (see http://www.aceaquatec.com). In situations where this particular device is used with a trigger, results from this study might not apply and it is possible that habituation can be delayed or partly prevented. However, the sound of this device is still produced in bursts instead of single isolated pulses and therefore carries some potential for habituation.

Pleasantness of sounds in marine mammals: Psychophysical factors influencing responses to anthropogenic noise

While no clear differential responses to the tested sounds were found in the captive trials deterrence ranges and the number of animals observed closer than 60 m differed across sounds types in the wild. In addition the number of seals within the overall observation area was lower after the sound exposure for the two new sounds. As mentioned in the introduction behavioural responses to an artificial sound which has no biological meaning to the animals can potentially be influenced by a variety of psychophysical factors of the sound. In humans sounds that have low tonality, high sharpness, high roughness and high loudness are perceived as unpleasant (Zwicker & Fastl, 1990). Particularly loudness is complex to estimate since it depends on stimulus intensity as well as on other sound features (e.g. loudness increases when signal bandwidth exceeds the cochlear filter bandwidth) (Zwicker et al., 1957). In the following discussion, sensation levels are used as a proxy for equal loudness contours while the other factors are evaluated qualitatively. The maximum sensation level that each sound type could cause at 1m distance was calculated by measuring the maximum difference between the composite hearing threshold (see appendix 1) and the referenced power spectrum of the sound type in 1/3 octave bins (from 100 Hz up to 24 kHz). Deterrence ranges were defined as the upper edge of the distance bin furthest away from the loudspeaker within which the number of animals was significantly reduced during sound exposure. Avoidance thresholds expressed in sensation levels were calculated by subtracting the measured transmission loss (see fig 8) from the maximum sensation level. The avoidance threshold in units of sensation levels therefore gives the sound pressure level in dB above the hearing threshold at which a sound causes a deterrence effect. Avoidance thresholds were also given as absolute received levels that were obtained from the sound field measurements. Table 3 summarizes the mentioned features for all tested sounds. It becomes obvious that while maximum sensation levels at 1m distance differed by about 20dB between different sound types; apart from one outlier (Square 500/530). Avoidance thresholds expressed as maximum sensation levels were roughly between 65 and 75 dB. This might indicate that some of the differences in deterrence range can be attributed to differences in sensation levels and therefore perceived loudness.

However, the data gives also evidence that psychophysical features other than sensation level are important. As mentioned earlier the new sounds were optimised

	White noise	**Sine 500**	**Sweep FM**	**Square 530/500**	**Airmar**	**Lofi-tech**	**Ace-Aqua-tec**	**Tere-cos**
Deterrence range [m]	**60**	**40**	**60**	**80**	**(40)**	**(60)**	**(60)**	**(?)**
Maximum sensation level (1m distance) [dB above hearing threshold]	108	92	100	96	110	110	111	107
Avoidance threshold								
Sensation level	**74**	**64**	**66**	**59**	**(79)**	**(75)**	**(74)**	(?)
SPL	138	144	144	135	144	138	138	?
Tonality	low	high	low	low	med	high	high	low
Roughness	low	low	high	high	low	low	low	med
Sharpness	low	low	low	low	low	high	med	high
BW effect on Loudness	high	low	med	med	low	low	med	high
Potential for habituation in the wild ?	low	low	low	low	high	high	high	high

Table 3: Comparison of psychophysical features of the tested sound types. The maximum sensation level was calculated as the highest value obtained when subtracting the hearing threshold from the composite audiogram (see appendix) from measured rms source levels in 1/3 octave bands. Discomfort threshold refers to the sensation level/SPL at the edge of the deterrence range.

to cause maximum roughness sensation and therefore potentially unpleasantness. The overall most effective sound type was one of the new supposedly unpleasant sounds (Square 500/530) causing deterrence ranges up to 80m. In contrast the control sound sine 500Hz caused deterrence effects up to 40m and white noise did the same up to 60m. However, more importantly Square 500/530 was able to deter seals at the lowest sensation level of 59 dB (see table 3) while both control sounds needed to have sensation levels of 74 and 64 dB to cause a similar deterrence effect (see avoidance thresholds expressed in sensation levels in table 3). Statistical evidence shows that the new sounds were more effective in deterring seals in 2 out of 3 response variables (number of animals in observation area <100m & deterrence range) while graphical evidence indicates that at least one of the new sounds (Sweeps) was also slightly more effective in a third response variable (no of animals< 60m). This would give evidence for the notion that roughness sensation might cause a perception of unpleasantness in seals as well as in humans. This may be surprising for scientists arguing for the uniqueness of human sound perception as a result of culture. However, given that according to Plomp & Levelt's (1965) theory of tonal consonance roughness and even consonance are mainly associated with cochlear filter bandwidth it seems a realistic result. Some evidence might point towards roughness sensation being shared by other marine mammals. Nowacek et al. (2004) demonstrated that right whales exhibited strong responses to alerting stimuli some of which were frequency-modulated at modulation rate that can potentially increase roughness but the animals more or less ignored playback of ship noise. While this is an interesting result it should be noted that whales were most likely highly habituated to boat noise. Therefore, only a comparison with similar artificial sounds that were unknown to the animal would have provided definite evidence for unpleasantness due to roughness. Some of the current seal scarers also share features that can contribute to unpleasantness (e.g. sharpness). Apart from sensation level and the other mentioned factors influencing pleasantness, responses could have been influenced by the fact that some seals might have experienced certain sound types in the wild and maybe had habituated to them (e.g. current ADDs). It is also puzzling that current acoustic deterrent devices differed particularly with respect to one device (Terecos) which did not cause a significant reduction in the number of seals at all. However, any conclusions with respect to current ADD sounds should be drawn with extreme caution since these sounds were only tested 6 times. This strongly decreased the likelihood for obtaining a significant result.

Although the data from the field are consistent with the assumption that certain features of pleasantness of sounds are shared by all mammals or possibly even many vertebrates I believe that several confounding factors were involved in this study making it difficult to draw definite conclusions. These factors include the fact that it was impossible to quantify behaviour on an individual level in field trials and a lower accuracy of quantifying avoidance responses in the field (analysis in 20m distance bins). Place preference experiments or two alternative forced choice experiments with captive animals would be needed to answer this question more definitely. However, given that the novel sounds proved equally effective compared to the seal scarers in experiment 1 and more effective than current ADD sounds in experiment 2 while causing lower sensation levels in odontocetes, it would be advantageous to test them in ADDs.

Avoidance & discomfort thresholds in seals and humans

In humans, discomfort thresholds have been measured using electro-physiological procedures by monitoring parameters that are indicative of stress (e.g. skin currents, cerebral cortex potentials) as well as by applying psychophysical methods. While psychophysical studies suggested values above 100 dB electro-physiological measurements clearly demonstrated that from a physiological point of view distress and discomfort start at sensation levels as low as 70 dB (Spreng, 1975). The avoidance thresholds expressed as the maximum sensation level at which animals avoided a certain sound type (table 3) ranged from 59-79 dB with an average value of 70 dB. However, seals may have had previous experience with seal scarers which might influence their avoidance thresholds. If data for seal scarers are removed average avoidance thresholds would be 66 dB above the hearing threshold for my experiments. This would mean that the avoidance threshold found in my experiments closely matches the discomfort thresholds obtained from physiological measurements in humans. Although this is speculative it could mean that the avoidance threshold reflects the onset of discomfort or distress in seals similar to the electro-physiological data for human subjects. The differences between psychophysical and physiological measurements in humans may in part be due to the fact that human subjects living in high noise habitats (like a city) underestimate perceived discomfort in spite of the fact that their body shows a stress response. With respect to the seal data it may be possible that stimuli exceeding the threshold by 70 dB are sufficient to cause physiological changes (e.g. stress) in an animal which consecutively lead to movement responses away from the sound source. However, more experiments on more species would be needed to investigate if onset of stress/discomfort in relation to the species' hearing threshold is a generic pattern within mammals.

Avoidance thresholds have been measured in captive harbour seals and harbour porpoises by analysing movement patterns (Kastelein et al., 2006; Kastelein et al., 2005, note that the authors called these discomfort thresholds). In harbour seals avoidance thresholds were 108 dB re 1µPa for sounds covering a frequency range between 5-15 kHz which the authors stated to be only 30 dB above the detection threshold at the given background noise level (Kastelein et al., 2006). The maximum sensation of this sound under quiet conditions would therefore even be below 50 dB. Similarly, the data for harbour porpoise that were tested in a small net pen suggests that the avoidance threshold is associated with sensation levels in the order of 50 dB (Kastelein et al., 2005). Avoidance thresholds calculated from my data are higher than those provided by Kastelein et al. (2006). In both of Kastelein's studies on captive animals only a very limited number of specimens (2 porpoise, 5 seals) were tested. In addition seals were not tested individually and could have therefore influenced each other (as pointed out by Southall et al. 2008). In contrast, the samples in my study were drawn from a pool of several hundred animals and were carried out over the course of a year. It is therefore difficult to say whether the results from the two captive studies by Kastelein et al. (2005, 2006) can be considered representative. Avoidance threshold in seals from the wild might be more representative and closer to the human data compared to Kastelein et al's (2005, 2006) experiments in captivity.

Marine mammal noise exposure criteria

When comparing the data from this study to severity ranking scales for aversive responses in the context of recently suggested marine mammal noise exposure criteria (Southall et al., 2008), the responses in the field trial would be at level 6 on a scale from 1-9. This means that an avoidance response of the sound source has occurred. In the captive trials the most extreme avoidance responses were found to habituate quickly and animals resume successful foraging after a few sound exposures. However, no quick habituation was observed in some response variables (e.g. dive time). Shorter dive times could lead to more frequent dives and therefore elevate metabolic costs of successful foraging and might therefore have to be considered problematic as well. The responses in the pool trials would be at level 5-6 at first (moderate changes in trained behaviours) but down to level 4 after the habituation process in the first playback session. The authors of the noise exposure review (Southall et al., 2008) concluded that responses of pinnipeds to non-pulses are "poorly understood" and no value can be given due to lack of and partly contradicting data. The data from this study could fill this gap and point towards levels of 135-145 dB re 1µPa in a frequency band between 500 Hz and 15 KHz. This would relate to maximum sensation levels between 60-70 dB with respect to level 6 responses. In conclusion, given that wild cetaceans have been shown to avoid areas where sensation levels roughly exceed 70 and 80 dB (see Johnston, 2002 and considerations in the introduction) the phenomenon of marked aversive responses starting at a sensation levels of about 70 dB might be more widespread among different taxa than originally thought. It may therefore be worth to consider the possibility that behavioural avoidance thresholds (>180 dB re 1µPa) suggested for odontocetes by Schlundt et al. (2000) and applied by the US navy (Anonymous, 2005) are too high.

Conclusions

In the light of the problem of potential hearing damage (see chapter 2) and the fast habituation process observed in this study, the use of high duty cycle seals scarers on fish farms seems problematic. Although animals habituated to all sounds when food motivation was high the new sounds were more effective in field trials and given their expected lower impact on *odontocetes* it may be worth testing these sounds in seal scarers if no better method is available. The data gives some indication that while sensation levels seem to be relevant for the strength of an avoidance response other features of a sound that influence loudness or general pleasantness are important as well. This is mostly marked by the fact that one of the new sounds based on the model of unpleasantness (maximised roughness) caused the strongest deterrence effect in spite of not having the highest sensation level. Roughness perception might therefore follow similar principles in animals as in humans making it unlikely to be a result of culture. However more experiments in controlled settings are needed to answer these questions related to “pleasantness” perception of sounds in animals. In general, animal experiments on phylogenetically distant taxa can be considered a powerful tool to investigate whether phenomena like musical consonance preference are a result of culture or physiology. Furthermore, all behavioural responses observed in this study are consistent with predictions obtained from human psycho-physiological studies. This might indicate that some processes relating to sound perception in seals or possibly other marine mammals might be a result of the general functioning of the cochlea rather than specific adaptation to the aquatic habitat.

The data can also be used to make predictions about impact of anthropogenic noise (e.g. industrial noise) on marine mammals. The avoidance threshold for seals has been shown to be in the order of 60-70 dB above the hearing threshold depending on sound type.

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Chapter 4

Functional aspects of grey seal underwater calls and their potential application in seal deterrence

Introduction

Eared seals (Otariidae) generally form harems on land and males monopolise females during the breeding season. In contrast, the majority of true seals (phocids) mate aquatically in an environment where resources and access to females are difficult to monopolize (reviewed in Cassini, 1999). Most phocid seals are known to produce one or several types of underwater calls. Calling behaviour is either particularly pronounced during or even limited to the breeding season (Thomas et al., 1983; Green & Burton, 1988a; Hanggi & Schusterman, 1994; Cleator et al., 1989). Gregarious, aquatically breeding seals of the polar regions seem to have adopted more complex vocal repertoires than their counterparts of the temperate regions. The Pacific population of harbour seals (*Phoca vitulina)* produces four different call types (Hanggi & Schusterman, 1994) and Scottish harbour seals only use a single simple call (with two subtypes) that consists of structured noise (van Parijs et al., 1999). In contrast, ice-breeding harp seals (*Pagophilus groenlandicus*) of the artic region have a repertoire of 16 calls including a variety of pulsed, tonal as well as more noisy calls (Mohl et al., 1975). The vocal repertoire of Antarctic ice-breeding Weddell seals (*Leptonychotes weddellii*) even amounts to an impressive size of 36 call types (Green & Burton, 1988a).

From an evolutionary point of view the two sympatric Scottish seal species represent an interesting case with respect to the differences in their mating system and underwater calling behaviour. Harbour seals breed on land throughout the vast majority of the species' distribution range (Sullivan, 1981). In contrast, several grey seal populations (*Halichoerus grypus*) still breed on pack-ice or ice floes and the species shows substantial adaptations for reproduction in a variable, arctic habitat. This is shown by the presence of white lunago in pups and a short lactation period (see Lydersen & Kovacs, 1999). In harbour seals underwater vocalisations are only produced by males and seem to be limited to the breeding season (Van Parijs et al., 2000a; Hanggi & Schusterman, 1994). Ice-breeding grey seals in Canada, however, have been reported to produce at least 7-8 different types of underwater calls with some evidence for females vocalising as well (Asselin et al., 1993). Similarly, Scottish grey seals produce 10 different underwater calls with observations supporting the notion of vocalising females. Interestingly, the 6 most common calls produced by Scottish grey seals around breeding sites were also found to be used around non-breeding haulout sites outside the breeding season (McCulloch, 1999). Traditionally, mating in grey seals has been regarded as solely terrestrial while harbour seals are considered to mate aquatically (Sullivan, 1981). However, it is entirely unknown whether ice-breeding grey seal populations mate aquatically or on land (Lydersen & Kovacs, 1999). Even in terrestrially breeding populations underwater copulations are possible (McCulloch, 1999) and might be more common than originally thought (Amos et al., 1995). Also, different individuals seem to employ different mating strategies (Amos et al., 1995). In land-breeding populations males which force underwater copulations are able to convey their genes, although they seem to have somewhat lower reproductive success (Lidgard et al., 2004). The individually lower reproductive success might however underestimate the role of aquatic copulations on a population level since a high number of individuals seem to employ satellite strategies and copulate underwater. This is supported by the fact that apparently 50-70% of the pups in grey seal breeding colonies originate from males that never show up on the breeding beach and are therefore not competing for females on land (Wilmer et al., 1999). This would mean that although on an individual level the strategy of monopolising females on the beach is more successful on the population the majority of pups seem to come from males that roam the water or hold underwater territories.

Underwater calling in phocid seals has been associated with a variety of different contexts and functions. These include male-male aggressive interactions, mate attraction, establishment of dominance hierarchies, territorial defence, a function similar to birdsong or a role in facilitating herd formation on ice (Thomas et al., 1983; Terhune & Ronald, 1986; Hayes et al., 2004a; Cleator et al., 1989; Green & Burton, 1988b). While it has been attempted to investigate the function of harbour seal (Hayes et al., 2004b) and Wedell seal calls (Thomas et al., 1983; Watkins & Schevill, 1968) through playback experiments knowledge of grey seal underwater vocalisations remains patchy and speculative (see Asselin et al., 1993). Underwater vocalisations in grey seals have first been described in two juveniles by Schusterman et *al.* (1970). These animals produced clicks as well as calls with a harmonic structure (humming calls and moans). Asselin et *al.* (1993) also reported tonal calls (moans), pulsed vocalisations (“knocks”, “clicks” and “trots”) and calls that have a pulsed nature followed by additional sharp up or down-sweep (“rups” and “rupes”).

Most of these calls fall into a frequency band between 100 Hz and 4-5 kHz. Similar vocalisations were found in Scottish grey seals with the addition of a few more call types e.g. a very low-frequency call with no energy above 200 Hz that consists of a short high repetition rate pulse trains leading to a seemingly harmonic structure (type 10 call, McCulloch 1999).

Since grey seals are major predators on some fish farms in Scotland (see Quick et al., 2004) improving the knowledge on grey seal underwater vocalisations would not only be desirable from a biological point of view but could also prove useful with respect to potential applications in acoustic deterrence. For example, if some calls are used in male-male competition and if it was possible to exaggerate certain call features that convey male dominance then playbacks of modified calls could be used to deter other males. In addition, it is not known if call types have functions other than male-male interactions or mate attraction The relatively large vocal repertoire compared to harbour seals and the occurrence of vocalisations outside the breeding season suggest that this might be the case. Non-mating related calls in marine mammals have been shown to serve a variety of different functions. Bottlenose dolphins (*Tursiops truncatus*) use distinct frequency-modulated whistles to convey individual identity information in order to maintain group cohesion (Tyack, 2000; Janik & Slater, 1998; Janik et al., 2006). Some terrestrial mammals have evolved sophisticated alarm call systems e.g. vervet monkeys (*Cercopithecus aethiops*) use functionally referential calls to warn conspecifics of a certain species of predator (Seyfarth et al., 1980). In birds alarm and distress calls are common and have been implemented in acoustic deterrence systems with varying success (see Bomford & Obrien, 1990 for review). In any case using natural calls of animals for an application in acoustic deterrence requires a thorough understanding of call function as well as knowledge on the type of behavioural response that can be elicited through playbacks. The primary goal of this study was to investigate if movement behaviour of grey seals can be modified by playbacks of conspecific calls. The secondary aim was to use these results to infer possible functions of grey seal calls with respect to evolutionary questions related to pinniped underwater communication.

Experiment 1: Behavioural responses of wild-captured grey seals to conspecific calls in a large pool

Methods

Experiments in captivity were carried out in a pool of 2.5 m depth and 40m by 6 m surface area. The pool was filled with sea water pumped in from the St Andrews Bay. Eleven grey seals were tested of which ten were females (5 adults, 5 juveniles) and one was a juvenile male.

The playback stimuli were

Control sounds

- white noise
- sine wave of 500 Hz
- harbour seal roars (this call was chosen since it represents a natural call that falls within a frequency band similar to grey seal calls. However, since there are no known social interaction between both species harbour seal calls should not be socially relevant to grey seals).

Grey seal calls (classification in brackets from McCulloch 1999; sonagrams of the 6 tested call types are shown in appendix 2)

- Moans (type 7): Calls with a harmonic structure and occasional frequency modulation; fundamental frequency ranges from 100-700 Hz; audible impression very similar to calls frequently heard in air when seals are hauled out.
- Rupe (type 5): Multiple element call with peak frequencies between 100 and 300 Hz; consists of two elements that are repeated up to 20 times; 1st element can be interpreted as a pulse or sharp downsweep while the 2nd element is tonal, longer and typically decreasing in frequency.
- Rup or “guttural rup” (type 1): Call is similar to rupe but tonal component/element is missing.
- Growl (type 9): noisy call; if harmonic structure present then it is associated with non-linear phenomena.

- Type 10: very low-frequency call with a harmonic structure that is however most likely due to the pulsed nature of the call (high repetition rate pulses); fundamental frequency ranging from 20-150 Hz.
- Knocks : pulsed call causing the audible impression of a knock against a wooden door; calls is broadband with energy up to 4 kHz; knocks are emitted in sequences of 1-5 calls.

Fish sounds: Selection of herring gas bubble release sounds and haddock spawning calls

Recordings were collected by McCulloch in 1999 at Scottish seal haulouts on the Isle of May during the breeding season and at the Tenstmuir haulout site outside the breeding season. Grey seal calls were digitized from analogue tapes. At least 10 examples of each call type were extracted to prevent pseudoreplication. However, only 6 suitable growl sounds could be extracted from the recordings. The -20 dB start and end point around the peak frequency of the loudest section or element of the call was defined. Then this section of all calls was normalised to the same average rms-level. This procedure resulted in calls causing a similar sensation of loudness in human listeners. A playback session consisted of a no sound treatment, the three control sounds (white noise, sine wave, harbour seal roar) and 7 test stimuli (moans, rupes, rups, growls, type10 calls, knocks, fish sounds). All playback stimuli were presented in pseudo-randomised order which was different for each animal. Four playback sessions were carried out with each individual over the course of at least 2 days. Playback sessions were separated by at least 3 hours. Each recorded example of a call type was never presented more than once to the same individual.

The experimental setup consisted of an underwater feeding station (see experiment 1 in chapter 2) placed at the side of the pool at 1.2 m depth, two underwater cameras, one aerial surveillance camera and the sound source and a J11 projector. The J11 sound projector was provided with a test certificate by Underwater Sound Reference DivNPT (US Navy) but the frequency response was also verified using a similar procedure to the one described for the Lubell transducer. It was found that the J-11 transducer produced even low-frequency components of calls (<200Hz) very well. The J11 transducer was deployed from a movable crane at a depth of approximately 1.5m. The distance between the underwater feeding station and the J11 transducer was between 7.5 and 8m. The visibility in the pool was never higher than 3-4m and often reduced to less than 2m. The loudspeaker was therefore invisible to a seal positioned in front of the underwater feeding station. The experimental pool was originally designed for diving physiology experiments and was permanently covered with 6m long and 1.5 wide wire panels that restricted the surfacing area for seals. With all panels closed seals could only surface under a plexi-glass pyramid (“breathing box”) that was normally used for respirometry measurements. All seals had previously spent some time in the pool and some seals had even spent one week under closed panels. For my experiments 4 wire panels were lifted, 1 in front of the feeding station, 1 at the position where the loudspeaker was lowered into the pool and 2 further away from the loudspeaker. Seals could also surface in the breathing box. The movement behaviour of seals was also restricted by a net extended over a length of 58 m parallel to the wall of the pool where the feeding station was located. The net was located at 1.5m distance from the wall. Seals could change between different sections of the pool by surfacing or swimming around the net. Seal behaviour was monitored with two underwater cameras, one placed 2 m away from the feeding station and one positioned to provide a good view of the sound source. Additionally an aerial camera was mounted on a 5m long pole in order to provide an overview of the whole pool area showing all four potential surfacing spots. All cameras were linked to a multiplexer and recorded as described in the experiment 1 of chapter 2. In addition an HTI hydrophone was deployed next to the feeding station to ensure that the playback was working properly and monitor received levels at the feeding station. The hydrophone was linked to one of the two audio channels (AV port) of the video camera to enable analysis of possible vocal responses in relation to the video streams. The rms-source level of the loudest section of the call selected by the -20 dB start and end points around the peak frequency was 135-137 dB re 1µPa. The peak to peak source level ranged from 145 to 155 dB re 1µPa depending on the sound type. Pulsed elements in rups and rupes had slightly higher p-p levels than the more tonal moans. Received levels at the feeding station were approximately 15-20 dB lower.

The seal was attracted to the underwater feeding station by lowering the edge of the feeding cup prior to the playback. Once the seal approached, the cup was lowered completely and the fish was taken by the seal. If the seal stayed close within view of the underwater camera a playback was started 30s after the seal took the fish. However, if the seal retreated e.g. towards the breathing box vanishing from the view field of the underwater camera the start of the playback was delayed until the animal positioned itself again in front of the feeding station and was visible in the view field of the underwater camera. Behaviour was monitored over 3 min following the start of the playback.

In the experimental setup the seal had the chance to either show an avoidance response by reatreating towards the breathing box (away from the loudspeaker) or approach the sound source. In order to describe movement responses an approach score was defined.

1 Turn towards speaker but no approach.
2 Swam towards playback source entering the “channel” between the net and the wall which lead towards the loudspeaker. However, the seal did not appear in the field of view of the underwater camera monitoring the sound source (since the camera was facing into the channel the seal must have turned back about half-way through the channel).
3 Approached and surfaced in the spot around the loudspeaker where the wire panel was lifted close to the playback source. However, seal did not appear in the field of view of the underwater camera monitoring the sound source.
4 Animal approached the loudspeaker closer than 1.5m. This means it could be observed on the underwater camera monitoring the sound source within the designated area.
5 Came close as in 4 but also touched the speaker with its muzzle.

A movement away from the sound source was also monitored and would have been categorised as -1 if the animal retreats in the channel but does not show up in the breathing box and as -4 if the animal showed up in the breathing box. However, playbacks of grey seal calls only elicited attraction or no clear movement responses. Therefore, an approach score is used to describe responses. To prevent pseudo-replication of the data by using multiple measurements for each individual seal median approach scores were calculated across the 4 playback sessions. Although there was no clear habituation effect visible across the 4 playback sessions most seals did only respond two or three times to each stimulus even for the call types that elicited the strongest responses.

In addition to the approach score, the time the animal spent close to the loudspeaker was measured. This was done by analysing videos from the underwater camera looking down at the loudspeaker and using natural marks on the pool wall to estimate an area of 1.5m around the loudspeaker. In order to compensate for any possible effect of habituation or playback order on response magnitude the maximum time each animal spent close the loudspeaker was used. Then median time spent close was calculated across all individuals that had exhibited a close approach. Close approaches were also analysed by life history data of the seals. Three categories were used: Pups (Juveniles) born in previous autumn captured at the breeding site

(Isle of May) which have probably rarely been in the water, 2.) Pups (Juveniles) born in previous autumn captured at a non-breeding (resting) haulout site (Abertay Sands /Tentsmuir). 3.) Mature adults. The plotted ratio of behaviours exhibited by seals in the three different categories was compensated by the amount of seals in each category (5 adults, 3 juveniles/pups from the Isle of May, 3 juveniles/pups from Tentsmuir). Finally, in order to measure how effective a sound type was in distracting seals from foraging, the mean time each animal spent within 1.5 m of the feeding station was calculated.

Statistical tests were calculated in SYSTAT 11.0. A repeated measured test was used for time spent close to the feeding station since values for all 11 individuals were available. However, this was not possible for “terms of time spent close to the loudspeaker” since not all individuals showed close approaches in response to all tested sound types.

Results

Grey seal calls: Approach score

Median approach scores differed significantly for the 10 different playback stimuli (Friedman test, F=48.521, p<0.0001). Playbacks of all tested calls tended to either initiate approach responses or did not cause any apparent movement response but none of the calls caused an immediate flight response away from the sound source towards the breathing box. There was no clear habituation effect in the sense that responses (e.g. approach score 4) did decline gradually from playback session 1 to 4. For instance, some animals responded to a certain stimulus in playback session 1, did not respond to the same stimulus in sessions 2 or 3 but showed a response in session 4. Except for two individuals, animals did not show an approach response more than 2-3 times to each stimulus. Only one individual, a juvenile seal, responded to a call type (moan) in all 4 playback sessions. The strongest median approach responses were clearly elicited by moans, rupes and rups (see fig 1). Post-hoc comparisons between all grey seal calls and the harbour seal control were carried out since harbour seal call are biological sound within a similar frequency band that should not be socially relevant to grey seals. Approach scores during playbacks of moans, rupes and rups were significantly higher compared to the playback of harbour seal calls (Friedman test with Bonferroni adjustment for multiple testing). However, there was no significant difference in approach responses between the harbour seal

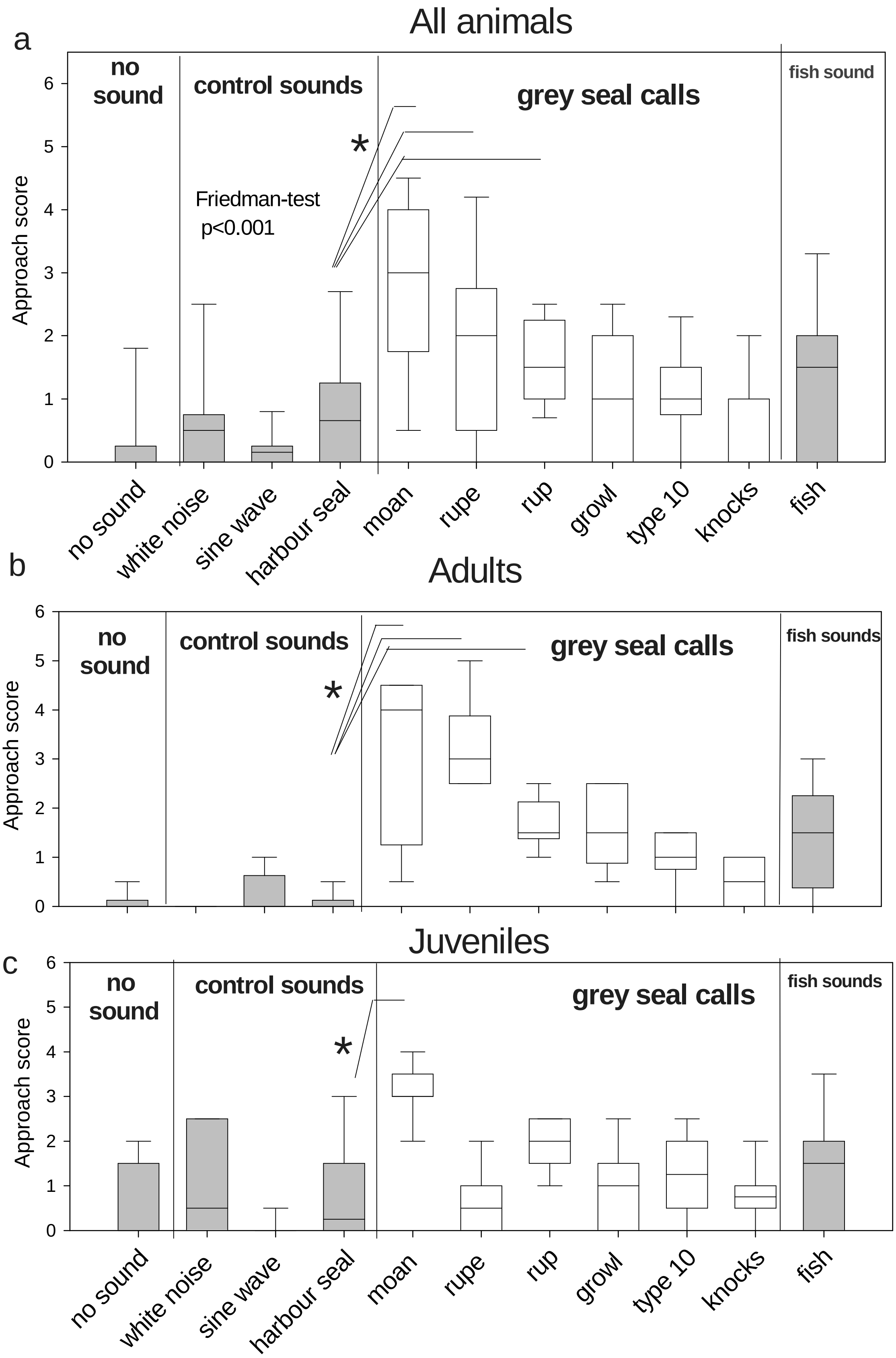


Fig 1: Approach score for all animals (a), adults (b) and juveniles (c). The box plots depict the median and 25 & 75 percentiles. Whiskers display the 5 and 95 % data range. Significant differences between the harbour seal control treatment and any of the grey seal calls are shown by asterisks.

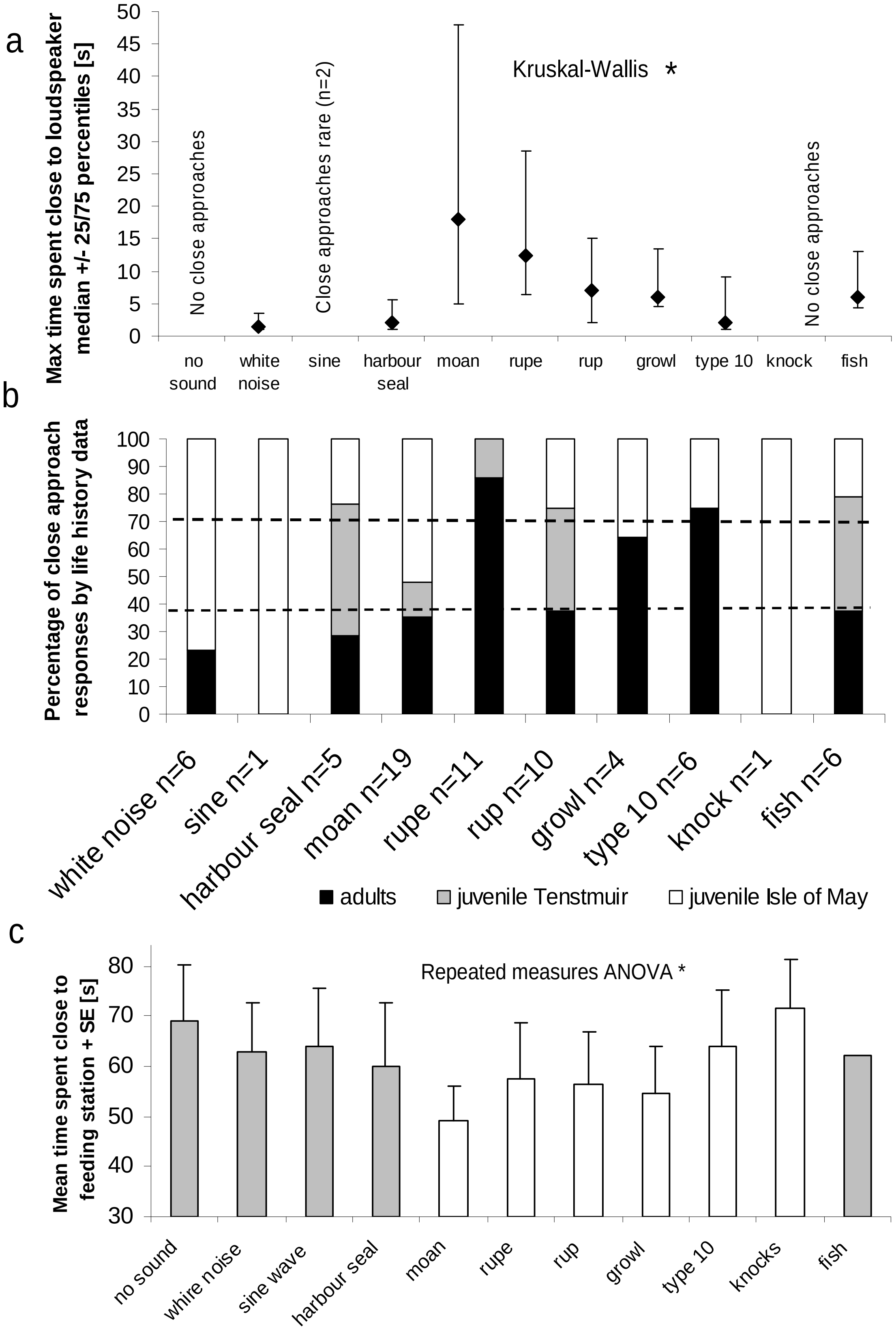


Fig 2: a) Time spent within 1.5m of loudspeaker after a close approach. The Kruskal –Wallis test was calculated over all sound types for which median values are displayed in the graph (n=8). , b) percentage of close approaches by life history data (overall number of close approaches per treatment is given as n). Dashed lines show ratios expected by chance level, c) mean time spent close to the feeding station as a measure of how successful seals could be distracted from foraging. The missing columns in a) and b) are due to the fact that animals either never showed a close approach or less than 1/3 of the individuals exhibited close approaches ("close approaches rare").

control and growls, type 10 calls, knocks after Bonefrroni adjustment of the significance level was applied. For approach responses a strong difference in behaviour between juveniles and adults was found (figure 1). In the 5 adults, approach responses were significantly stronger in response to moans, rupes and rups compared to the harbour seal control. However, in the 6 pups only playbacks of moans elicited approach scores that were significantly higher than those during the harbour seal control. Moans, rupes and rups also frequently caused adult females to inspect the loudspeaker closely with their muzzle (approach score 5). This behaviour involved pushing the transducer gently with the nose and exhibiting extended exploratory behaviour towards it (see fig 3). Some of this behaviour could maybe also be classified as “social” e.g. when animals swam close circles around the loudspeaker probing it from different directions. Investigative behaviour and touches of the loudspeaker with the nose were exhibited during 8 playbacks of moans by 6 different individuals, during 6 playbacks of rupes by 4 different individuals and 3 times in response to rups by 3 different individuals. This behaviour was only shown once by one individual in response to a growl, type 10 call or a harbour seal call respectively. The behaviour was never shown in response to any of the other stimuli. Investigative behaviour was also much more common in adult females compared to juveniles: 5 out of the 6 animals that touched the loudspeaker in response to playbacks of moans were adult females. All individuals that showed investigative behaviour and touched the loudspeaker in response to playbacks of rupes and rups were adult females.

Figure 3: Approach score “5” response typically exhibited by mature females. The seals investigated the loudspeaker with their nose from different directions, swam circles around the loudspeaker and stayed close for an extended time period.

Grey seal calls: Close approaches to the loudspeaker

Close approaches (approach score 4 or higher) occurred frequently in adult seals but to some extent also in juveniles (see fig 2b). Close approaches occurred most frequently in response to playbacks of moans (19 times by 9 different individuals), rupes (11 times by 6 individuals) and rups (10 times by 9 individuals). Approaches were occasionally also caused by playback of the control sounds, however, approach events in response to these stimuli were much less common (see “n” for each playback stimulus in figure 2b). The maximum time each individual spent close to the loudspeaker having exhibited a close approach (score 4 or higher) was compared across playback stimuli (fig 2a). Only those stimuli were included that had caused a close approach at least once in one third of the tested seals (see fig 2a). These stimuli were white noise, harbour seal roar, moan, rupes, rups growl, type 10 and fish sounds. The median time seals spent close to the loudspeaker differed significantly between playback stimuli (Kruskal-Wallis test, p=0.016, H=17.29, df=7, note df is less than 10 since not all sound types caused close approaches). This might show that different playback stimuli caused different levels of “interest” in the seals. Graphical evidence in figure 2a shows that playback of moans & rupes and to a lesser extent rups caused animals to stay much longer in the vicinity of the loudspeaker than any other playback stimulus (fig 2a). Particularly the scatter towards higher values shows that longer stays were more common in response to playbacks of moans and rupes. The longest time a seal stayed in the immediate vicinity of the loudspeaker was 100 seconds after playback of a rupe. Animals never spent any time close to the loudspeaker during the no sound control.

Figure 2b summarizes close approach events for different groups of seal reflecting different levels of experience with underwater calls in the wild. Since all mature females tested in this experiment were pregnant they can be considered to have experience with underwater calls in the wild (having most likely participated in several breeding seasons). In contrast juveniles that were captured on the breeding site (Isle of May) have rarely or never been in the water and may have therefore never or rarely been exposed to underwater calls. Juveniles captured at Tentsmuir must have swum at least once from the breeding site to Abertay Sands and have therefore stayed at a resting haulout site for a while. They can therefore be expected to be slightly more likely to have been exposed to underwater calls. Figure 2b shows that close approaches to rupes were almost exclusively exhibited by adults. Similarly, adults were more likely to approach growls and type 10 calls compared to juveniles. However, the overall number of approaches in response to these two call types was low (n=4 and 6) compared to moans, rupes and rups (n=19, 11 and 10). Approaches in response to the artificial control sounds (white noise, sine wave 500 Hz) were mostly exhibited by juveniles captured at the breeding site (Isle of May).

Behaviour around the feeding station

The time each seal spent close to the feeding station provides information on whether playbacks of grey seal calls can be used to modify behaviour of foraging seals e.g. to lure them away from a foraging spot. The mean time each individual spent within 1.5m of the feeding station during each treatment is shown in fig 2c. A significant difference in the mean time spent close to the feeding station was found between the 10 playback stimuli (repeated measures ANOVA, $F_{1,10}=1.9$, $p=0.045$). Playbacks of moans caused the strongest reduction in the time each seal spent close to the foraging spot. However, even when moans were played seals still spent 27% of the monitored time (3min) close to the foraging spot compared to 38 % during the no sound control. The result shows that the success rate of luring a seal away from the feeding station is relatively small.

Responses to fish sounds

The median approach score calculated across all individuals was significantly higher for playbacks of fish sounds compared to the harbour seal control (Friedman test F=7, p=0.008 1, df=1). This indicates that seals showed consistent approaches in response to fish sound although median approach scores were lower than in responses to grey seal calls. Figure 1 b and c show that approaches were also exhibited by juveniles. Juveniles captured at the breeding site showed proportionally less approaches to fish sounds but the pattern does not seem to be different to the harbour seal control playback (fig 4 c)

Vocal responses

Only 1 out of 11 seals vocalised frequently in the pool. There were two more possible instances when another seal might have produced a rup or rupe but the sounds were very faint and difficult to discriminate from background noise. The one seal that vocalised frequently was a mature female which generally seemed to be less habituated to the captive environment than other individuals (this animal was more easily scared by humans). This animal frequently produced long sequences of rups and some rupes. It also sometimes produced type 10 calls. On one occasion a moan was identified. Vocalisations were not restricted to the 3min observation periods following the playback but were also emitted in the breaks between playbacks. No quantitative analyses of call type similarities and vocal responses have been carried out so far. The data showed however that female grey seals are capable of producing rups, rupes and type 10 calls.

Experiment 2: Grey seal behavioural responses in the field

Methods

The experimental protocol was the same as that described in chapter 3 experiment 2 except for a few differences that are described in the following section. Experiments were conducted around in the area described chapter 3 in experiment 2 within a similar time period (March 2006-May 2007). I attempted to achieve as much of a temporal and spatial separation of both experiments as possible. During most of 2006 grey seal call playbacks were carried out around the haulout sites on the inner sand bars at Tentsmuir forest while artificial and startle sounds were played at the remote sandbars in the river Tay (March 2006-July 2006). However, from August to September 2006 and in spring 2007 some playback sessions using artificial sounds and grey seal calls had to be carried out around the inner sandbars. In that case, grey seal calls were always played first and a 30min break was introduced after the end the last 15min observation period before a playback session of the louder artificial sound started. Furthermore, if two haulouts were present on the inner sandbars (usually separated by 300-600m distance) the haulout site was changed after completion of grey seal call playbacks. Also, playback of loud artificial or startle sounds was never followed by playback of grey seal calls on the same day. These procedures were implemented to ensure that no carry-over effect was present. Overall, 15 no sound control observation periods, 12 playbacks of moans and 10 playbacks of rupes, rupes and harbour seal calls were included in the analyses. Some playback had to be excluded due to disturbance of the haulout by walkers on the beach scaring an abnormally high amount of seals into the water.

The playback protocol was the same as in chapter 3 (experiment 2). A playback consisted of 5min pre-playback observation, 5 min sound exposure and a 5 min post observation period followed by a 15 min break before the next playback started. No sound or call type was tested more than once on each day. While all monitoring equipment and procedures where the same as in experiment 2 in chapter 3 the playback setup and the playback source were different. All sound stimuli were played through a J11 transducer. The rms-source level of the loudest element of a call defined by its -20 dB duration was adjusted to 141-143 dB 1µPa. The peak to peak source levels of the loudest element ranged from 150-156 dB re 1µPa. The J11 transducer was deployed in mid-water hanging on a chain from a 70cm-diameter floating buoy. The transducer was deployed at a depth of 1-1.5m. A rope was attached to the floating buoy to adjust the distance between playback boat and loudspeaker. As a result of tidal currents and wind the buoy with the J11 transducer drifted away from the boat and the rope was tied to the boat once a distance of 10m was reached. This was done to enable grey seals to approach the loudspeaker closely avoiding any possible deterrence effect of the boat. The direction of the buoy was monitored with a handheld compass during the beginning of each of the 5min observation periods. In contrast to the experiment in chapter 3, seal surface positions were monitored up to distances of 110m, allowing for the additional 10 m distance of the source from the boat. Bearings to all surfacing seals were measured with a handheld compass. Therefore, the distance (length of rope) and direction of the loudspeaker (handheld compass) as well as all distances and bearings to the logged surface positions of seals were known. This data was then used to calculate the distances between each seal and the transducer using simple trigonometric equations.

Sighted grey seals were classified by age class and sex into 4 different categories. Mature male grey seal were distinguished from mature females by the shape and length of the muzzle (concave versus more convex shape; long versus short muzzle). This difference is obvious for mature males and females. However, subadult males can be difficult to identify and there is some risk of classifying subadults males as females. Therefore, only clearly identifiable males and females were counted in the appropriate categories and a third category of “unidentified” animals was used in case of any doubt. The fourth category included juveniles and was restricted to animals that had substantially smaller heads reflecting the head size of pups that were born in previous autumn. This category did therefore not include subadults but must have largely consisted of pups aged 5-11months.

Results

Movement behaviour of seals in response to playbacks

The distribution of grey seals within the observation area was not influenced by the general experimental treatment (no sound control) or playback of harbour seal roars (see fig 4). There was no significant difference in seal numbers in any of the distance bins between the pre-playback, playback and post playback observation periods for the no sound control and playback of harbour seal calls (fig 4). In contrast, playbacks of all three tested call types attracted animals from more remote parts of the observation area towards the loudspeaker. Changes in seal distribution between pre-playback, playback and post-playback observation periods within each 20m distance bin were tested with repeated measures ANOVAs ($p<0.05$ was considered significant; see asterisks versus n.s. in fig 4). Playbacks of moans and rups led to an increase of seal numbers at distances of 20-40m from the transducer. For moans this increase appeared to be mostly due to seals arriving during the post observation period. Playbacks of rups lead to a twofold increase in seal numbers during the playback. However, rupes clearly caused the strongest attraction responses. Seals numbers increased significantly even in the closest distance bin 0-20m from the sound source where usually very few seals were seen. Furthermore, seal numbers increased significantly in the distance bin from 40-60 m and a non-significant trend towards higher seal numbers was also seen in the distance bin from 20-40m (see fig 4). Analysis of 70 % of the videos from the underwater camera monitoring the area around the transducers did not suggest that seals ever approached the immediate vicinity of the loudspeaker.

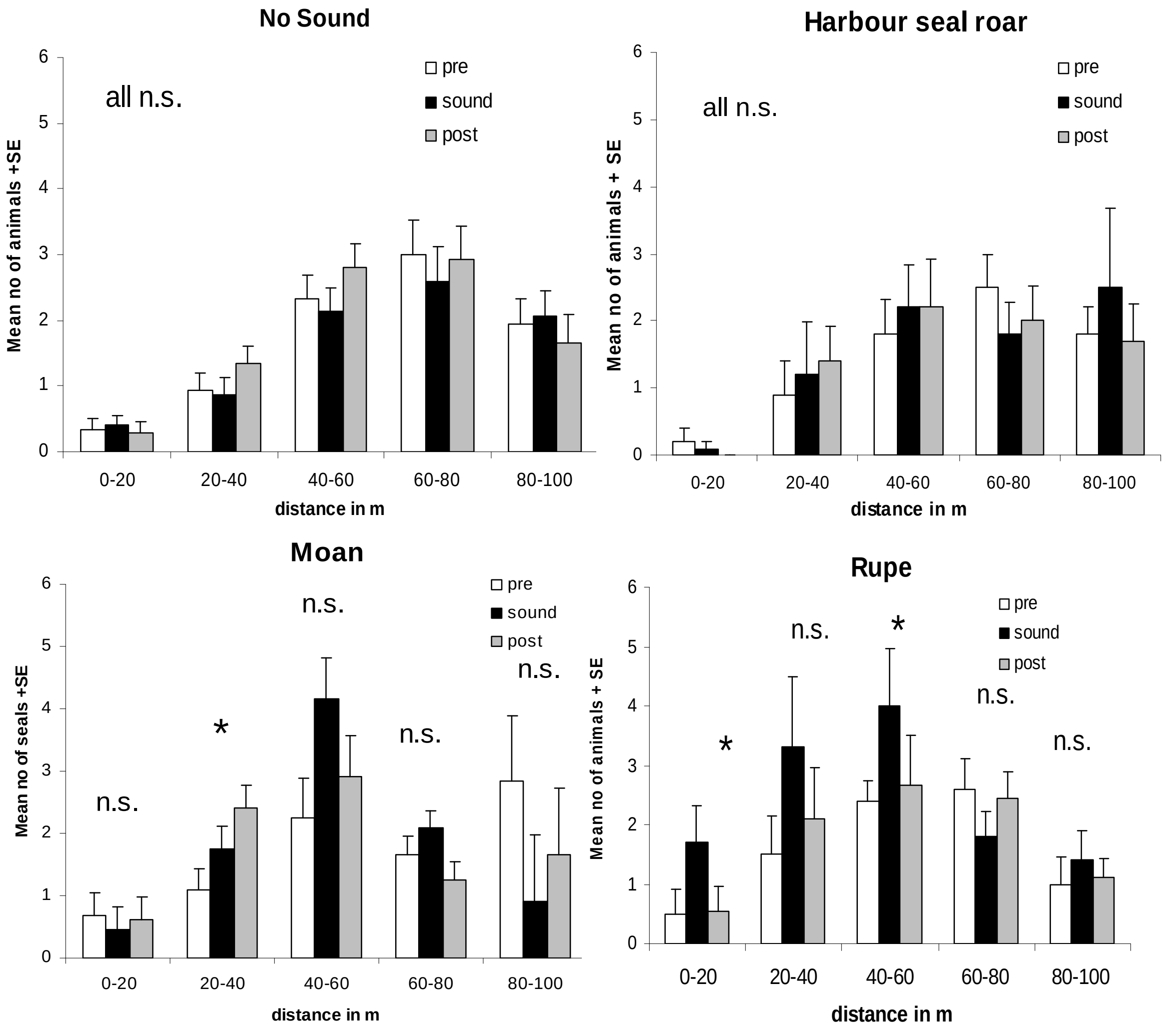
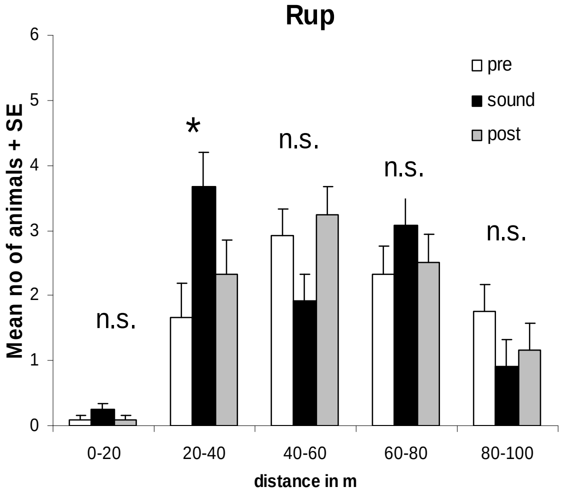


Fig 4: Mean number of sighted seals for the five tested treatments in the playback experiments at the haulout sites. Significant differences in seal numbers between the three observation periods (pre, sound, post) are shown by asterisks. Seal numbers did not change during playback of the harbour seal calls or the no sound control. In contrast significantly more seals were seen in at least one of the distances bins during playbacks of moans, rupes and rups.



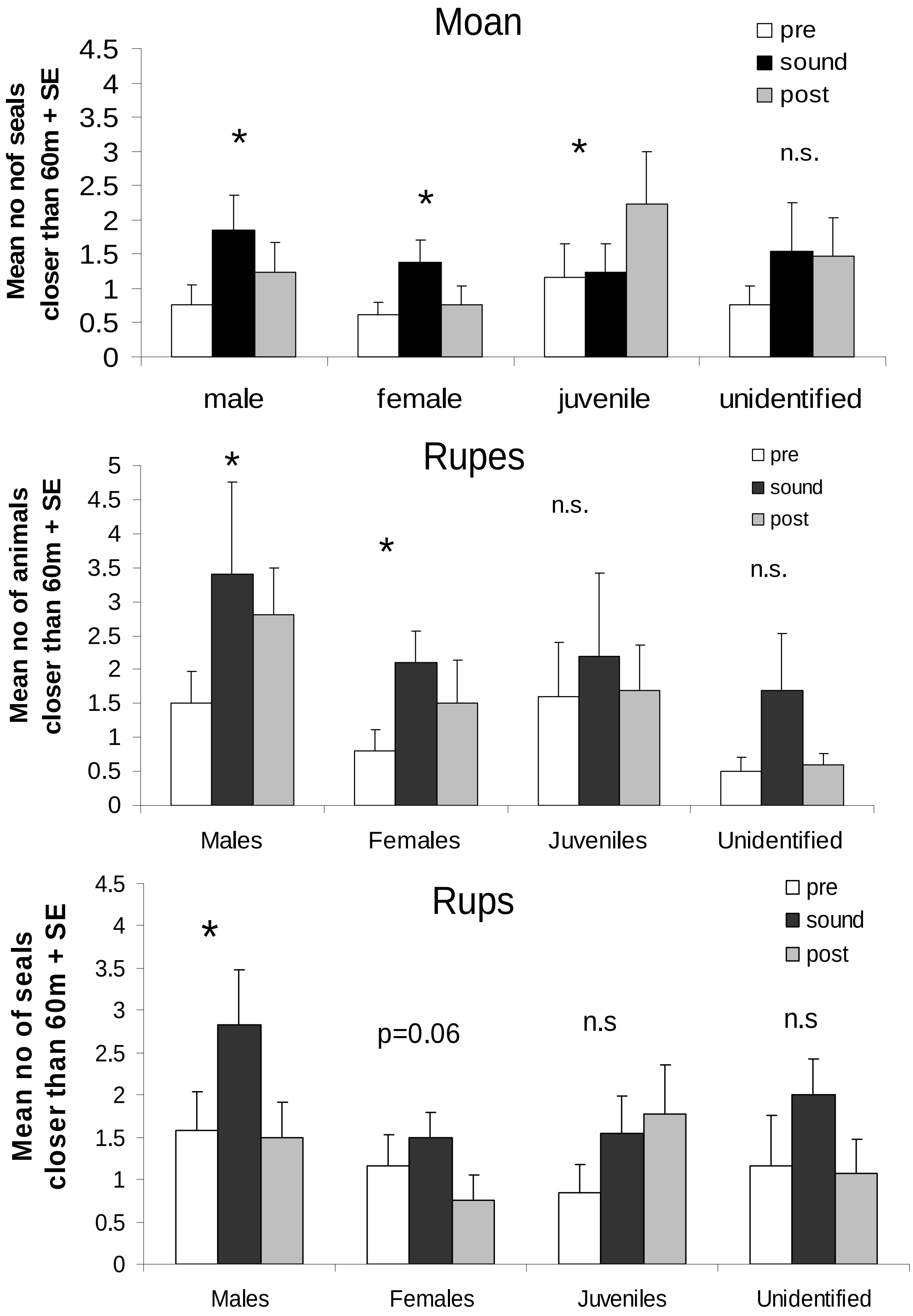


Fig 5: Approach responses by sex and age class. Graphs show the mean number of mature male & females, juveniles and unidentified animals that were sighted within 60m of loudspeaker before, during and post sound exposure. Repeated measured ANOVAS indicate significant changes in seal numbers. However, due to the data being split in 4 groups and possible interactions between the categories with respect to problems of correctly identifying animals the statistical power of the tests can be expected to be low.

Movement responses by sex and age class

The mean number of mature males and mature females, juveniles and unidentified animals was compared across the three 5min observation periods (pre, sound, post) within an area closer than 60m from the loudspeaker (fig 5). In general, numbers of identified males were slightly higher than females. Figure 5 shows at least a slight increase of seal numbers during or after sound exposure for all four categories of seals in response to all tested grey seal call types (fig 5). However, the strength of the responses as measured by changes in seal numbers varied between playback stimuli, sex and age categories. Also, differences as tested by repeated measure ANOVAs across the three observation periods were not significant for all groups and playback stimuli (significance level of $p<0.05$). Playbacks of moans resulted in a significant increase of males and females during sound exposure. Juvenile seal numbers increased significantly during the post observation period. Similarly, playback of rupes resulted in a strong increase of males and females within 60m of the sound source. The increase in juveniles and unidentified animals was not significant. Rups seemed to have a stronger attraction effect on male seals while the changes in numbers of females can only be considered a slight trend ($p=0.063$). In general it seemed that adult seals exhibited their movement responses faster than juveniles. While numbers of mature females and males reached the maximum during sound exposure juveniles only seemed to arrive in the area closer than 60m during the post observation period (see moans in fig 5).

The data showed that playbacks of all three call types caused approach responses in adult female and male grey seals while rups seemed to attract mostly males. Juveniles were only attracted by moans although playback of rups and rupes caused a slight but non-significant increase during the post observation period. Numbers of unidentified seals were also higher during the playbacks but differences were not significant.

Discussion

Evaluation of the data

Animals tested in captivity were naïve to sound exposure before the experiment and were habituated to captivity. However, there were some differences in baseline behaviour e.g. 2 individuals were more likely to spend time in the breathing box which they knew from previous physiology experiments to be a safe place. Differences in baseline behaviour might explain some of the variation in the responses to the playbacks. All seals were tested alone but some individuals (mostly juveniles) had been in the pool together prior to the experiment. This means that, in theory, some animals might have heard underwater calls in the pool before, while others did not which could have influenced responses to the playbacks. However, vocalisation rates in captive grey seals housed alone or in small groups in captivity seem to be generally low. Vocal behaviour of two juveniles was monitored when held together in the pool. Only one possible call was detected in 8h hours of recordings. Vocal behaviour of 3 animals was also monitored during diving physiology experiments when the wire panels at the water surface were all closed and animals were isolated in the pool for up to a week. Several days of recordings revealed only 2 possible instances of vocalisations.

Another potential problem is that although there was no clear habituation effect , animals respond differently in different playback sessions. I believe this has to do with playback order and the fact that a high number of different stimuli (10 and the no sound treatment) were tested. If for example, one of the call types that generally elicited strong attraction responses was played very late in a playback session it was less likely to elicit a response. Similarly, if an animal showed a strong response to a certain call type in session 1 this could have led to a lack of response in session 2 but responses could have recovered over night. Since averages and median values were calculated over all playback sessions and playback order across individuals and sessions was randomised these effects should equal out. However, the statistical power for detecting differences between control sounds and certain call types may have been reduced by the lack of a response in some sessions.

In terms of the field trials the classification of grey seals into the 4 categories might be a possible source of error. The interpretation of consistent changes in the category of unidentified animals is problematic since identification is likely to depend on surface time and distance the seal was away from the observer. It may be possible that seals that had been categorised as “unidentified” at higher distances from the observer were classified as “female” or male” after having approached the loudspeaker. With respect to responses within each of the 4 categories one should therefore look at the general proportion of animals rather than whether differences were significant or not. The general pattern suggests that approach responses occurred in females and males while the group of unidentified animals might have been subject to losses or gains of animals depending on observation conditions. Given that sex ratios in grey seal population are generally biased towards females it is surprising that more males than females were detected. While this could be due to an observer bias a more likely explanation is that males are less scared by the presence of the boat and therefore more likely to approach (males are generally known to be quite inquisitive). If however females are more hesitant to approach the area around the boat this would in turn mean that the experiment had low power for detecting approaches of females to the loudspeaker. In conclusion the data seem to be sufficient to conclude that both sexes were attracted by playbacks of moan and rupes.

As mentioned in chapter 3 the analysis by distance also poses some problems since it means that the pre sound exposure distribution of seals influences the likelihood of obtaining a statistically significant result. However, since distribution of animals was known from the no sound control and the pre periods for all call types distance bind are useful to test for changes of seal numbers around the sound source.

Can playbacks of conspecific calls be used to modify grey seal movement behaviour?

A review of a variety of different studies testing acoustic deterrence methods in birds and mammals revealed that playbacks of conspecific alarm and distress calls were generally more successful than artificial sound with no biological meaning (Bomford & Obrien, 1990). In marine mammals, playbacks of killer whale calls have been shown to cause strong avoidance responses in seals (Deecke et al., 2002) and cetaceans (Cummings & Thompson, 1971; Fish & Vania, 1971) while playbacks of conspecific's calls have never been tested in the context of acoustic deterrence in a marine mammal. However, humpback whales (*Megaptera novaeangliae*) have been shown to exhibit differential movement responses when being exposed to playback of songs versus social calls (Tyack, 1983). Only a few underwater playback experiments monitoring movement behaviour have ever been carried out in seals: playbacks of roars to harbour seals resulted in aggressive approaches (Hayes et al., 2004b; Thomas et al., 1983) while playbacks of trills and chirps to Weddell seals did not cause strong attraction although seals approaching a breathing hole were diverted to the loudspeaker (Watkins & Schevill, 1968). Similarly, my data shows that none of the tested grey seal calls caused a deterrence effect in captive and wild grey seals which would have been detected as a reduction of seal numbers around the boat in the field experiments and as a retreat towards the breathing box in the captive trials. However, playback of moans, rupes and rupes caused significantly stronger approach responses than a biological control sound (harbour seal roar). In the captive experiment where moderate food motivation was simulated with an underwater feeding station, playbacks resulted in a significant but small decrease of the time seals spent close to the foraging spot. It might therefore be feasible to influence movement behaviour of grey seals by playback of conspecific calls, however, the effect is likely to be short-term and small. It therefore seems unlikely that it would be possible to prevent an animal from approaching a foraging spot for more than a few minutes. This is shown by the fact that seals did not stay longer than 1-2min close to the loudspeaker after an approach but usually returned to the feeding station quickly.

One possible application might be to exploit the strong and predictable attraction responses to lure a “rogue” animal (e.g. a seal foraging on farmed salmon) into a trap and then relocate it. In addition, the responses found in this study could be used to temporarily distract seals from a foraging spot but this is unlikely to last for a very long time. In conclusion, acoustic deterrence or attraction through playbacks of grey seal calls is unlikely to be very successful although playbacks of moans, rupes and rups might prove a useful tool for certain specific applications.

Response to fish sounds

The responses exhibited to fish sound were not particularly strong but consistent across individuals. Even juveniles showed approaches to fish sounds, however, the difference in the median approach score between the harbour seal control and fish sound playback was less pronounced for juveniles (see fig 1b and c). Passive listening has been previously suggested as a mechanism for prey detection in pinnipeds (Schusterman et al., 2000) and there is some evidence that even echolocating odontocetes may respond to fish sounds (Gannon et al., 2005). Gannon et al. (2005) showed that bottlenose dolphins turn towards a loudspeaker and apparently only echolocate when fish calls were played back. The latter aspect of the study does however need to be looked at with extreme caution since the dolphins might have simply not pointed their narrow echolocation beam towards the hydrophone before the fish sounds were played and therefore no clicks were detected prior to the playback. This means that the result cannot count as evidence that echolocation is not the main mode of prey detection in dolphins. However, the study showed that passive listening is a realistic option for prey detection in marine mammals. The lack of stronger responses in my experiment might have been partly due to the fact that animals had to balance the benefits of approaching a potential prey item and staying close to a known foraging spot (feeding station). My data provides some evidence that passive listening for prey calls might be employed as a foraging strategy in pinnipeds.

Possible functions of grey seal underwater calls

The main findings of this study with respect to possible functions of grey seal underwater calls can be summarized as follows: 1.) Captive isolated grey seals exhibited instant approach responses to moans, rupes and rups while other calls caused only occasional attraction responses. Responses were more stereotypic in mature females compared to juveniles. Juveniles were more likely to show occasional approaches to biologically meaningless, artificial control sounds and only showed consistently higher approach scores in response to moans. Mature females exhibited extended exploratory behaviour and stayed in the vicinity of the loudspeaker for a longer time when moans, rupes and rups were played back. Vocal responses in captive seals were rare. 2.) In the wild, females and males showed approach responses to moans and rupes while mostly males were attracted by rups. Again juveniles only seemed to be attracted by moans. 3.) All mentioned behavioural responses were exhibited outside the breeding season in a non-breeding context.

Most of the suggested functions for underwater calls in phocid seals are somehow related to mating or breeding. For instance, the vocalisations of aquatically mating harbour seals might serve in male-male competition in a lek-type breeding system (Hayes et al., 2004b; Boness et al., 2006). Alternatively, in other areas or further away from haulout sites, some male harbour seals might also defend underwater territories (Van Parijs et al., 2000b). Bearded seals (*Erignathus barbatus*) seem to use vocalisations to defend underwater territories but some individuals also employ satellite strategies like roaming (Van Parijs & Clark, 2006). In Weddell seals, some underwater calls are likely to function in territorial defence while others seem to be used to signal aggression, threats or submissive behaviour (Thomas et al., 1983; Watkins & Schevill, 1968). Given the sequence structure, behavioural context and the big repertoire size, vocalisations of Weddell seals have also been considered to resemble song (Green & Burton, 1988b). McCulloch (1999) provided limited evidence for territorial underwater behaviour of male grey seals around a breeding site and showed that some call types in grey seals are emitted in non-random sequences possibly suggesting some role similar to song. One possibility is that grey seal calls might be related to breeding and are important in either male-male aggressive interactions or mate attraction. Taking it one step further, sequences of calls could also serve a dual function in repelling other males from intruding into a territory and attracting mates, similar to birdsong (see Collins, 2004). If some but not all vocalisations resemble song, one could expect that responses to these two stimuli to be different as it was shown for singing humpback whales (Tyack, 1983). Previous data on ice-breeding grey seals showed that vocalisation rates peak during that time of the breeding when copulations and social interactions are most common (Asselin et al., 1993). The responses shown by female grey seals in my experiments might be consistent with mate attraction but since they were exhibited outside the breeding season this is unlikely to be their sole function. The strong approach responses exhibited by males without any signs of aggressive behaviour towards the loudspeaker make it unlikely that moans, rupes and rups are primarily used in aggressive male-male interactions. In birds, for example, replacement of a territorial male by a loudspeaker playing back songs has been shown to repel other males and delay the reoccupation of a territory (Falls, 1988; Krebs et al., 1978). Control territories where males had been removed but no playbacks were carried out were reoccupied faster. My data for moans, rupes and rups is therefore inconsistent with a function in territorial defence similar to birdsong since no repellent effect on male grey seals was found in the field. However, the field experiment was carried out around a non-breeding site outside the breeding season and therefore the lack of aggressive responses could also be due to the context. Since playbacks attracted males without any signs of aggression it seems less unlikely that rupes, rups and moans are largely used in male-male interactions or territorial defence. However, such a function cannot be ruled out for type 10 calls and growls during the breeding season. The presence of female vocalisations in grey seals is also not necessarily consistent with the standard concept of song or mate attraction. In birds, female song is considered the exception rather than the rule, however, in some species females sing commonly and female song might serve a variety of functions ranging from territorial defence to coordination of breeding activities (Langmore, 1998; Krebs et al., 1978).

In the following section I try to suggest possible functions for each call type based on data from the literature and my findings. Analysis of underwater footage by McCulloch (1999) revealed production of rups by a male which tried to force an involuntary underwater copulation with a female (female tried to swim away and bit the male). I analysed underwater footage recorded by Bob Burville (see http://www.youtube.com/user/bburville) showing production of a single, isolated rup during an aggressive interaction of two pups. Rups may therefore be aggressive in a certain context but the fact that they caused attraction responses in both of my experiments shows that they could also function as some more general contact calls.

The case for rupes as an affiliative signal or possibly a contact call is stronger. Asselin et al. (1993) suggested that grey seal rupes might be primarily produced by females and function in female-female interaction or male-female interactions. While female production of rupes has been shown by one of the tested seals in experiment 1 there is also evidence that rupes are produced by males (McCulloch 1999). Further analysis of footage by Burville (see above) showed production of a single rupe in an affiliative context during a female-male interaction (possibly courtship). Rupes caused the strongest attraction responses in mature females (time spent close to loudspeaker) in captivity and were successful in attracting seals of both sexes in the wild.

It should be noted that both rups and rupes are generally produced in sequences which are highly variable in length and repetition rate (McCulloch 1999). In rupes, the more tonal element also varies in duration, frequency structure and frequency modulation. Although this is a purely speculative suggestion, it may be possible that this variation could in part determine the meaning of the call e.g. sequences involving fast repetition of rups or rupes could be aggressive while slow repetition rates may be affiliative. In conclusion the behavioural responses observed in my experiments are more supportive of rups and rupes signalling some kind of affiliative context possibly being some kind of more general contact call or some role in mate attraction.

Production of underwater as well as amphibious moans has been previously reported in two captive juvenile grey seals during social interactions (Schusterman et al., 1970). Schneider (1974) reported moans to be produced by captive grey seals of both sexes when animals were interacting, swimming in coordination, or when they were competing for food. In my experiments, a moan was the only call type that caused strong responses in juvenile seals. In contrast to other calls, moans are commonly produced in air and therefore relatively naïve juveniles (e.g pups captured at breeding site) are likely to be familiar with them. In my experiment on captive seals, moans generally caused the highest median approach scores. This call may therefore function in courtship or mate attraction during the breeding season or as a general contact call to negotiate affiliative or aggressive encounters of seals around haulout sites.

The functions of the other call types that did sometimes (growl, type 10) or never (knocks) elicited attraction responses are more dubious. Approaches in response to growls and type 10 calls were significantly higher than the harbour seal control before a Bonferroni adjustment was applied. Schneider (1974) mentioned growls to be associated with agonistic behaviour in captive grey seals. In two captive leopard seals (*Hydrurga leptonyx*) (subadult male and mature female), growls that were somewhat similar to the growls in grey seals were more likely to be associated with aggressive rather than defensive behaviours (Rogers et al., 1996). The audible characteristics of growls as well as type 10 calls might also subjectively imply some role in agonistic interactions. Growls and type 10 calls may therefore be used in agonistic close encounters of seals which could explain why only some mature seals approached the loudspeaker while other seals might have chosen to avoid a potential conflict. Knocks with a short rise-time and explosive character were also reported from ice-breeding grey seals (Asselin et al., 1993). In Weddell seals, pulsed calls, probably similar to knocks, might be associated with threats (Watkins & Schevill, 1968). It is therefore possible that knocks might function as a threat signal in very close aggressive encounters. Given the distance between the loudspeaker and the seal at the start of each playback this might explain the complete lack of responses in any of the tested seals in experiment 1.

Low-frequency underwater calls in pack-ice breeding harp seals have been associated with long-distance communication which might play a role in herd formation in a highly variable habitat (Terhune & Ronald, 1986). Terhune & Ronald (1986) also reported that breeding herds could be detected up to a distance of 30km with individuals possibly being either in direct or indirect acoustic contact (e.g. through links between several individuals). Extending the ideas of Terhune & Ronald (1986) I suggest a “haulout initiation hypothesis” as an additional function for moans, rupes and possibly rups. In this context “haulout initiation” refers to both the annual formation of breeding herds in ice-breeding populations but might also be important for colonisation of new breeding or even general resting haulout sites in rapidly growing populations. Calls might play a role to establish an underwater communication network (see Janik, 2005) which could lead to consecutive initiation of a haulout in a variable pack ice habitat. As has been shown by my data, these call types elicit strong attraction responses. Initially, animals that are spread out might only be able to directly hear a few other callers but through several links such a network could extend over large areas. Therefore, if each animal would exhibit an approach response towards a caller this should lead to the formation of aggregations within the network. These aggregations would develop into large acoustic targets if animals keep vocalising and therefore attract more animals from even further away. If seals would evaluate these sound sources and always approach the loudest target then aggregations should develop in only a few locations. In a more complex scenario it would be possible that a seal only responds vocally to a caller if it is in the vicinity of a potential haulout site (e.g. at the ice edge or near to the ice edge) but it would remain silent if it was alone at sea. If this is the case then underwater communication networks would converge towards a suitable haulout or breeding site. This haulout initiation hypothesis could also explain the different calling behaviour in harbour seals where breeding haulout sites are formed in stable habitats on land. In harbour seals, underwater calls are as far as we know largely produced by males exclusively during the breeding season. Female harbour seals do not seem to vocalise much and most importantly females are not attracted by male calls (Hanggi & Schusterman, 1994; Hayes et al., 2004b). This difference in behaviour might suggest that the evolution of harbour seal underwater communication was primarily driven by male-male interactions. In contrast, some grey seal underwater calls may have evolved to establish communication networks that function in haulout initiation on pack ice. The difference in calling behaviour between sympatric harbour and grey seals might therefore be explicable by the different evolutionary past of both species.

Possible perceptual learning effects

Vocal production learning is a rare ability among mammals and seems to lack entirely within our closest relatives, the primates (see Janik & Slater, 1997). In contrast, vocal production learning mimicking human speech has been convincingly shown in harbour seals (Ralls et al., 1985). In grey seals, only call usage learning has been demonstrated to date (Shapiro et al., 2004). The data from my experiments do not relate to vocal production learning but might hint at some basic learning process on the perceptual side which would not be surprising in a taxon that seems to be vocally quite versatile. In seals, there is some indication of perceptual learning processes for mother-offspring recognition in air (Charrier et al., 2001) and in birds there is good evidence for cultural transmission of song preferences (Riebel et al., 2002). Median approach scores in response to the apparently meaningless biological control sound were higher in juveniles compared to adults. Close approach responses to artificial control sounds were almost exclusively exhibited by juveniles captured at the breeding site. These animals might have rarely or never entered the water before capture. Differences in behavioural responses might be a result of learning or simply ontogeny. However, the fact that pups that were captured at a normal haulout site and the breeding site were both tested at a similar age but showed somewhat different behaviour might point towards a learning process. It is possible that juveniles have some kind of genetically determined “acoustic curiosity” leading them to show general approaches to any sound type. This baseline behaviour might then be altered by gaining experience with certain call types e.g. animals might selectively habituate to certain sounds (see Deecke et al., 2002 for a possible an example of selective habituation in seals). Alternatively, associative learning processes might enhance responses to certain call types. The latter would be similar to so called “action-based learning” on the production side which has been argued to be a widespread phenomenon among songbirds (Marler & Nelson, 1993). This form of learning involves selective reinforcement of production of certain song types from a bigger repertoire through social interactions. Action-based learning might be supported by the fact that the only call type that caused consistent approaches in juveniles was the moan, a call which juveniles commonly produce themselves in air and to which they were exposed at the haulout sites (in air) as well as in the test facility after capture. The described effects should, however, not be over-interpreted since sample sizes for each of the three groups was small making it difficult to test differences statistically.

Conclusions

The first underwater playback experiment on grey seals showed that seals exhibit clear attraction responses to some conspecific calls, namely moans, rupes and rups. The results suggest that these calls might be used as some general contact calls e.g. around haulout sites or in mate attraction . Given that these low-frequency calls cause strong attraction responses they might also have an additional function in order to establish underwater communication networks that mediate haulout formation. Growl, type 10 calls only caused occasional approach responses in mature female animals and might be used in close agonistic encounters between seals. Although some calls were successful in distracting seals from a foraging spot the effect was small and did not last very long. Therefore using playbacks of grey seal calls for “acoustic distraction” e.g. to lure animals away from a fish farm is unlikely to be successful. Playbacks of grey seal calls may, however, prove a useful tool to trap and relocate “rogue” seals that inflict damage on fish farms or fisheries.

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Chapter 5

The acoustic startle reflex in *phocid* seals: An initiator of extreme behavioural responses to anthropogenic noise

Introduction

The acoustic startle response (ASR) is an obligatory reflex that is elicited by sudden, short duration, short rise-time sound stimuli. The ASR is present in a variety of vertebrate species including humans and involves the interruption of ongoing behaviour patterns and the initiation of protective motor-patterns (Landis & Hunt, 1939). These motor-patterns typically involve an involuntary flexor contraction as indicated by sudden neck or body twitches. The ASR also induces physiological changes e.g. an increase in heart rate (Korn & Moyer, 1966) although cardiac responses are not necessarily evidence for startle (Tovote et al., 2005). In rodents startle responses can be reliably elicited by short rise-time stimuli that exceed the hearing threshold by more than 80-90 dB (Fleshler, 1965; Pilz et al., 1987; Davis, 1984). In order to elicit the reflex, a stimulus needs to reach its maximum intensity within 16ms of its onset, otherwise even a further increase of sound pressure up to over 140 dB re 20µPa will not trigger the reflex (Fleshler, 1965). In rats the startle threshold follows the hearing threshold at an average difference of about 87 dB with minima coinciding with the most sensitive hearing range (Pilz et al., 1987). The probability of reflex elicitation and response magnitude depend primarily on stimulus intensity (Pilz et al., 1988), rise-time (Ison, 1978) and to some extent on stimulus bandwitdh (Blumenthal & Berg, 1986). Startle response magnitude also increases as a function of stimulus duration but only up to durations of 6-8ms. This shows that integration times for the startle reflex are substantially shorter than the integration time of the rest of the auditory system (Marsh et al., 1973; Fleshler, 1965; Blaszczyk, 2003).

The startle response is mediated by a simple oligo-synaptic reflex arc located in the lower brainstem with the caudal pontine cochlear nucleus (PnC) forming the crucial sensory-motor interface (Koch & Schnitzler, 1997). In spite of the simple reflex-like nature, startle response magnitude/amplitude can be influenced by a variety of different factors in addition to the main ones mentioned above. A well known phenomenon in startle reflex modification is pre-pulse inhibition (PPI): In PPI response magnitude is reduced as a result of the presentation of a sub-threshold stimulus preceding the startling stimulus with lead times of 10ms-500ms (Stitt et al., 1976; Ison & Reiter, 1980; Ison & Hammond, 1971; Plappert et al., 2004; Stitt et al., 1973). In contrast, sub-threshold stimuli with lead times shorter than 10ms have the opposite effect leading to pre-pulse facilitation, an increase in response amplitude (Ison et al., 1973; Plappert et al., 2004). Furthermore, individual differences in anxiety state (Plappert et al., 1993), conditioned fear (Brown et al., 1951) and increased levels of background noise lead to sensitisation of startle response magnitude (Hoffman & Fleshler, 1963b).

While startle response magnitude is subject to long and short-term habituation, response latencies tend to be subject to sensitization i.e. they shorten over the course of several exposures (Pilz & Schnitzler, 1996). If the startle reflex influences the probability of eliciting flight responses there are in theory three possible scenarios how flight or avoidance responses might change over time: a) flight responses could be correlated with response magnitude and therefore be subject to long-term habituation, b) flight responses could be associated with the decrease in startle latency and therefore be subject to sensitisation, and c) flight responses could be independent of both or first follow one and then the other. It may also be possible that the strength of an avoidance response depends on one of the mentioned factors or is for example a function of startle amplitude. Schnitzler & Pilz (1996) hypothesised that sensitisation with respect to response latencies facilitates the efferent startle pathway down from the caudal pontine reticular nucleus (PnC) to the motor-neurons while habituation of response magnitude is mediated by changes in the afferent pathway. They also speculate that shortening of latency may facilitate motor behaviours like escape responses. However, no published data on behavioural follow-up responses associated with the startle reflex are available for any free-ranging mammal. While this is hardly surprising given that the majority of studies on the startle reflex are motivated by questions related to the neuronal basis of sensory-motor integration and simple learning behaviours (Koch & Schnitzler, 1997) this gap is severe with respect to the evolutionary function of the reflex. There are some anecdotal observations that rats either show “freezing” behaviour or a larger-scale directional flight responses in natural settings when exposed to startling stimuli (Pilz, 1984). It was suggested that the startle response may serve a function in facilitating or preparing a flight response e.g. in a predator avoidance scenario (Pilz & Schnitzler, 1996).

Small marine mammals can be considered an interesting model species for studying behavioural follow-up responses as well as startle thresholds as they possess sophisticated underwater hearing (Au et al., 2000; Southall et al., 2005) and are subject to noise pollution by pulsed anthropogenic sounds (Richardson et al. 1995). These include seismic shooting, airguns (used for oil exploration), military sonar systems or echo-sounders all of which produce high source level stimuli with short rise-times potentially capable of eliciting startle (see Richardson et al., 1995 for review on anthropogenic noise sources). In deep-diving beaked whales mass standings have been linked to naval exercises in which mid-frequency military sonar systems were used (Fernandez et al., 2005; Simmonds & Lopezjurado, 1991; Frantzis, 1998). Stranded animals showed gas bubble lesions that are consistent with decompression sickness (DCS) suggesting that lethal effects of sound exposure might have been a result of changes in diving behaviour (Jepson et al., 2003). In spite of the fact that most anthropogenic noise sources produce potentially startling stimuli at close ranges the startle reflex has been largely neglected in the marine mammal literature. More than 1500 articles have been published on the mammalian startle response over the last 50 years (keywords “acoustic startle response”, PubMed search), however, none got cited by any of the recent reviews on the impact of anthropogenic noise on marine mammals (e.g. Southall et al., 2008; Nowacek et al., 2007) although some bear potential relevance for it (e.g.Fleshler, 1965; Korn & Moyer, 1966; Tovote et al., 2005; Pilz & Schnitzler, 1996) .

A startle reflex, if present, might also be harnessed for marine mammal deterrence systems which would require less noise production than in current systems. Given that previous studies on rodents showed that the startle threshold seems to be parallel to the hearing threshold (Pilz et al., 1987) it would be possible to choose a specific frequency band and therefore create a stimulus that would exceed the startle threshold in a certain group of species up to a certain distance from the sound source but not in other species (e.g. pinnipeds versus *odontoces*, see chapter 2). The startle response therefore also holds potential to improve target-specificity of an acoustic deterrence system.

This study aimed to investigate whether the startle reflex is present in seals and what behavioural follow up responses are associated with the reflex in wild and captive free-ranging animals. This is also expected to shed light on the evolutionary function of the reflex. In addition, an investigation of startle reflex thresholds in water should provide information on possible adaptations as a result of aquatic hearing. I also investigated whether a startle pulse can act as an unconditioned stimulus in a classical fear conditioning paradigm which might be useful in deterrence applications and would be important for conservation issues.

Experiment 1: Startle reflex and behavioural follow-up responses

Methods

Seven grey seals and one harbour seal were tested in the experiment. Three of the grey seals were adult females, four were juveniles. Three of the juveniles were females and one was a male. The harbour seal was an adult male. The age of the juvenile grey seals ranged from approximately 7 -11 months at the start of the experiment. Tests were conducted in the same pool and with the same setup as experiment 1. The playback stimulus was a 200ms long noise pulse with a rise time of 5 ms. The peak frequency of the signal projected through the loudspeaker was 950 Hz. The -20 dB bandwidth spanned approximately two to three octaves with the average -20 dB power points being at 450 Hz and 1.9 kHz. The sound pulse was synthesized as a white noise pulse and then shaped

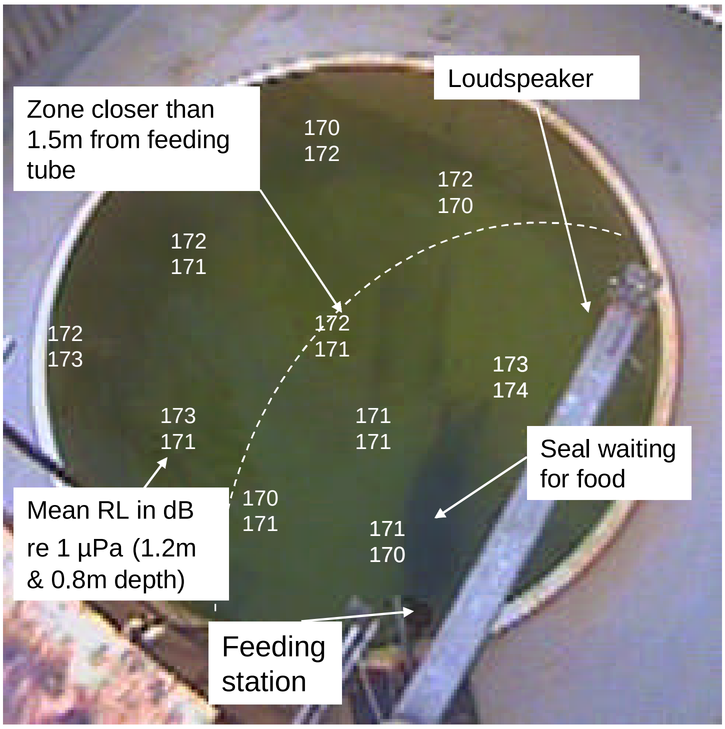


Figure 1: Experimental setup (experiment 1).The values shown in different sections of the pool give average received levels at 1.2 and 0.8m depth respectively.

through filtering processes (Butterworth filter). The startle pulse was always paired with a substantially weaker pre-sound to test whether the startle pulse can act as an unconditioned stimulus in a classical fear conditioning paradigm. The pre-sound was a 3-Hz-frequency modulated 1.2s long sine wave pure-tone. The sweeps caused by the frequency modulation covered a frequency range from 700Hz to 1.3 kHz. The pre-sound ended 2s before presentation of the startle pulse to avoid pre-pulse inhibition (PPI).

Received levels were measured at 0.8m and 1.2m depth and for four positions close to the tube also at 0.4m depth (animal spent most time deeper than 1m). The received level at the typical position of the animal’s head in front of the feeding station was between 170 dB and 171 dB re 1µPa. Measured received levels in the pool only ranged from 170 to 173 dB re 1µPa (rms). The received level of the pre-sound was between 125 dB and 130 dB re 1µPa.

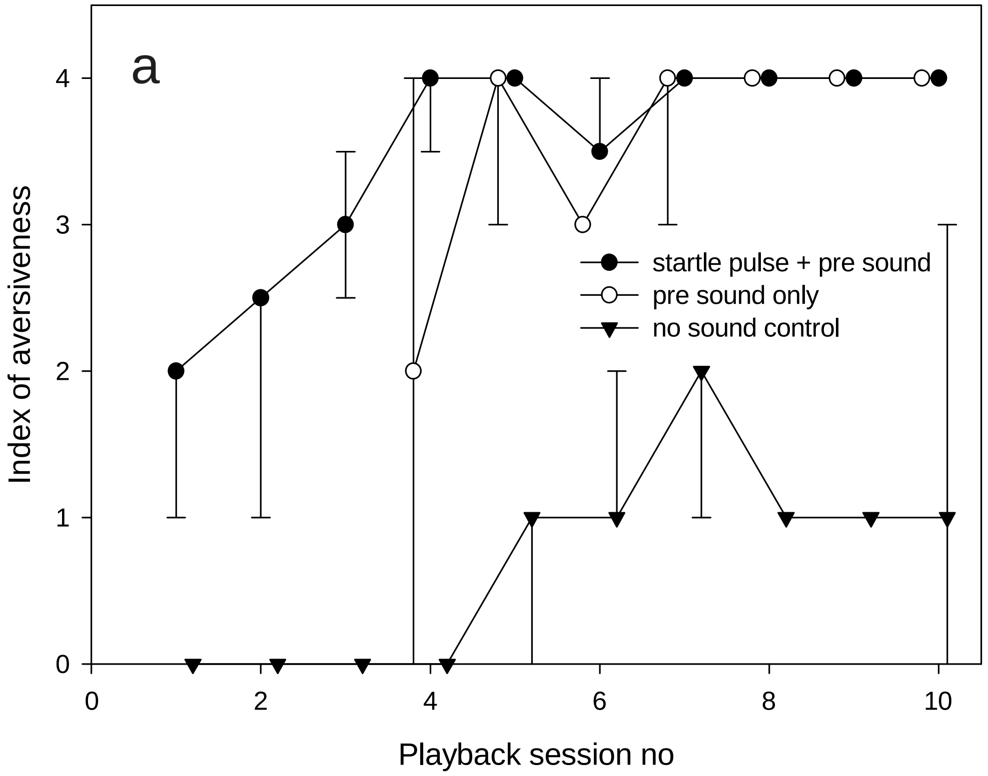
The experimental protocol, the response variables that were measured and the monitoring equipment (video & hydrophone) were the same as in experiment 1 in chapter 3. However, behaviour was monitored over 3 minutes instead of just one minute. The occurrence of clearly visible neck twitches or whole body muscle contraction was also monitored (as a sign of startle) as a response variable.

A playback session consisted of three or four treatments in the following sequence:

- no sound control
- startle pulse preceded by pre-sound
- startle pulse preceded by pre-sound
- after third playback session: pre-sound without startle pulse

The last of these treatments was only applied in playback sessions 4 to 10 but not in the first three sessions. Each 3min observation period was separated by a 5min pause. The term treatment refers to the three experimental conditions “no sound”, “startle pulses preceded by pre sound” and “pre sound only” during the 3min observation periods. As can be seen in the list above each treatment was applied once per playback session except for the “startle pulse preceded by pre-sound”

Animals that startled



Animals that did not show any signs of startle

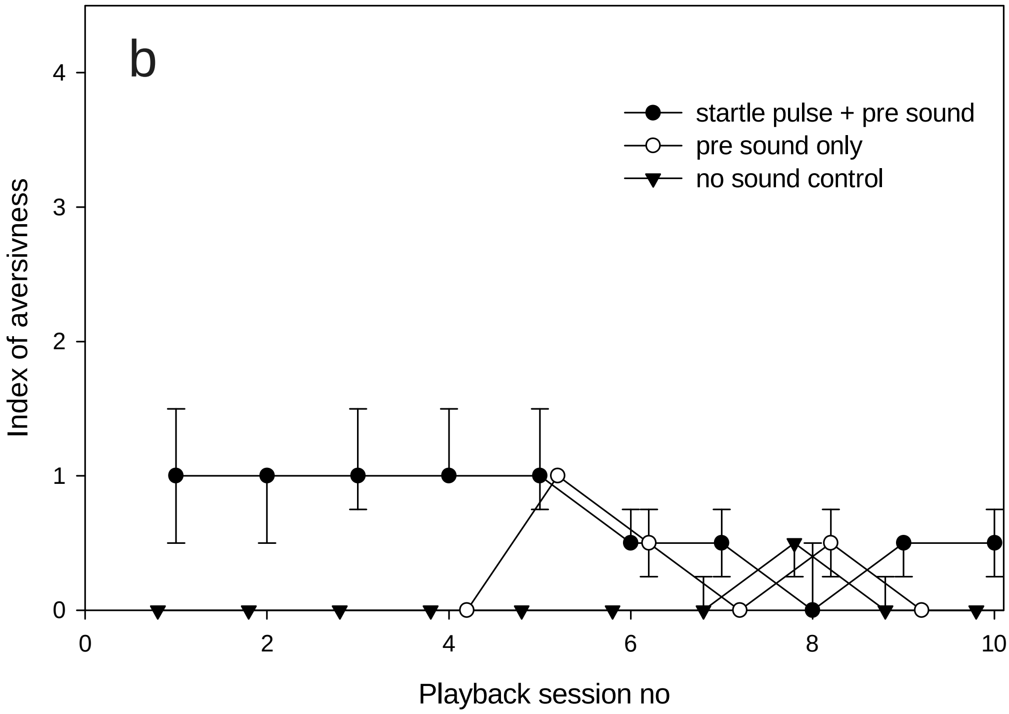


Fig 2: Index of aversivness for animals that startled (a) and animals that did not show any signs of startle (b). Data is shown as median +/- 25 and 75 percentiles. Aversive responses increased over time in animals that startled and animals sensitised to the experimental situation (see responses during no sound control). In contrast, in animals that did not show any signs of startle aversive responses were low and declined even further towards the end of the experiment.

which was carried out twice. The data from the two 3 min observation periods for the startle pulse were averaged. Each treatment started when the animal positioned itself voluntarily in front of the feeding station. If the animal stayed closer than 40cm for at least 30s after the first startle pulse a second startle pulse was presented. Also, if the animal left the feeding station but approached it again within 40 cm within the 3 min observation period, a second startle pulse was presented. Since no more than two presentations were given within the 3min observation period the maximum duty cycle for the startle pulses was 0.002 %. This responsive mode nature of the playbacks was done since it could potentially be used in acoustic deterrence systems leading to a reduction in duty cycle if a sound would only have to be produced if an animal stays close to the food source. A total of 10 playback sessions were conducted over the course of at least 3 days for each animal. Each playback session was separated by at least 20 min break and after two playback sessions a recovery time of at least 3 hours was given before a new playback session started. Data were analysed separately for animals that showed clear neck twitches (signs of startle) in the first two playbacks of experiment 1 and those who did not show any signs of muscle contraction.

Data were tested for normality (Kolmorgorov-Smirnov test) and if necessary transformed to achieve a normal distribution (“dive time” and “time close” for both groups of animals and “dive time” for the animals that did not startle). Variables were transformed using $\text{Log}_{10}(x)$. Non-parametric statistic and repeated measures ANOVAs were calculated in SYSTAT 11 (SAS) and general linear models were calculated in JMP 4 (SAS). The logistic regressions were computed in Sigma Plot 2002. This included a test for normality and autocorrelation of residuals.

Results

Index of aversiveness

The behaviour of the eight seals in this experiment fell into two broad categories. The majority of the seals (n=5) showed a clear indication of a startle response (e.g. neck twitches) while three animals did not show any signs of a startle. In the 5 individuals that showed signs of startle avoidance responses as measured by the index of aversiveness increased dramatically over time (fig 2a). The median index of aversiveness showed a strong and highly significant increase from playback session 1 to 10 in the startle treatment (Spearman rank correlation; $p=0.002$; $r^2=0.70$). This can be taken as evidence for sensitisation to the sound. By the end of the experiment all five animals showed an immediate flight response and left the water immediately after hearing the startle sound. A similarly strong response was caused by the "pre-sound only" treatment (see fig 2a) indicating that the seals learnt to associate the pre sound with the startle pulse. This is also supported by a comparison of the median index of aversiveness for the five animals that sensitised in the 4th and 10th playback session: A significant difference between the treatments "startle pulse+ pre sound" and "pre sound only" was found for the 4th playback session (Mann-Whitney U, U=21.5, p=0.043). However, both sounds always elicited the maximum aversive response (level 4) in playback session 10. This shows that the pre-sound had become as aversive as the startle sound as a result of a fear conditioning process. In the last three playback sessions some animals turned away from the feeding station and retreated immediately after the cup was lowered even if no sound was played. Given that they never showed this behaviour in the first 7 playback sessions this might indicate that they developed a general aversiveness against the place where they were exposed to the startle pulse. In contrast to the behaviour described for the seals that startled, responses of the three animals that did not show any signs of a startle reflex decreased from playback session 1 to 10 (fig 2b, Spearman rank correlation, p=0.006, r^2=0.63). This gives evidence for habituation. However, aversive responses in these animals were never particularly strong which can be seen by the fact that the median index of aversiveness was never higher than 1 for all playback sessions.

Time spent close to feeding station, haulout behaviour and diving patterns

General linear models (GLM) were conducted for the response variables "time spent within 1.5m of feeding station", haulout behaviour and dive time for both groups of animals separately (see table 1). Reactions of grey seals to the "startle pulse", the "no sound control" and the "pre-sound only" treatment are shown in figure 3. The model for the time spent close to the feeding station for the animals that sensitised was highly significant explaining 60 % of the variance (GLM, $F_{23, 111}$=9.12, p<0. 0001, r^2_{adj}=0.60). The factor with the strongest influence was treatment (i.e. startle pulse, no sound or the pre sound only). The second most important factor was playback session number (ranging from 1 and 10). This means that the behaviour of each animal changed over the course of the experiment and given the graphical evidence (see fig 3) this can be interpreted as sensitization in the sense of an increasing difference in aversive responses between sound exposure and the control treatment. While there was some individual variation in the general behaviour of "time spent close" the interaction term of individual and treatment was not significant showing that all individuals responded in the same stereotypic way to the sound. As shown in figure 3, animals that sensitised spent almost no time close to the feeding station by the end of the experiment in the startle treatment as well as when the pre-sound was presented alone. In the last playback session they also minimised the time close to the feeding station during the no sound control showing that previous experience lead to a strong aversion against the foraging spot even without any sound being presented. The model for haulout time was also highly significant (GLM, $F_{23, 111}$=8.27, p<0. 0001, r^2_{adj}=0.56) and showed that again treatment was by far the most important factor (see table 1). The second most important factor was playback session number which shows that animals changed haulout behaviour over time. Sound exposure never caused haulout behaviour in the first playback session but haulout times started to increase dramatically in the third playback session. In all following sessions the animals spent most time hauled out on dry land rarely entering the pool. The GLM for dive time yielded similar results (GLM, $F_{23, 111}$=12.4, p<0. 0001, r^2_{adj}=0.55), however, individual variation in diving patterns was stronger although individuals all behaved in a similar way to the sound exposure (interaction treatment x individual not significant). Playback number also had a strong effect on diving behaviour which from graphic evidence (fig 3) can be interpreted as sensitisation.

	Animals that startled						**Animal that did not show signs of startle**			
	Time close		**Dive time**		**Time hauled out**		**Time close**		**Dive time**	
Covariates	p	F	p	F	p	F	p	F	p	F
Treatment	**<0.0001**	29.8013	**<0.0001**	18.4951	**<0.0001**	32.9629	**0.0133**	4.6306	**0.0086**	5.1365
Individual	**<0.0001**	8.2706	**<0.0001**	40.2398	**0.0288**	2.8116	**0.0002**	9.8453	**<.0001**	47.558
Playback session	**<0.0001**	8.9398	**<0.0001**	6.6922	**<0.0001**	9.6711	**0.0431**	2.0953	0.4869	0.953
Treatment x Individual	0.0534	1.995	0.0756	1.8478	**0.0465**	2.0528	**0.0315**	2.8365	0.1769	1.6333

Table 1: Results of the GLMs for time spent close to feeding station, dive time and haulout time for both groups of seals. P-values for variables that had a significant influence on the response variables are shown in bold.

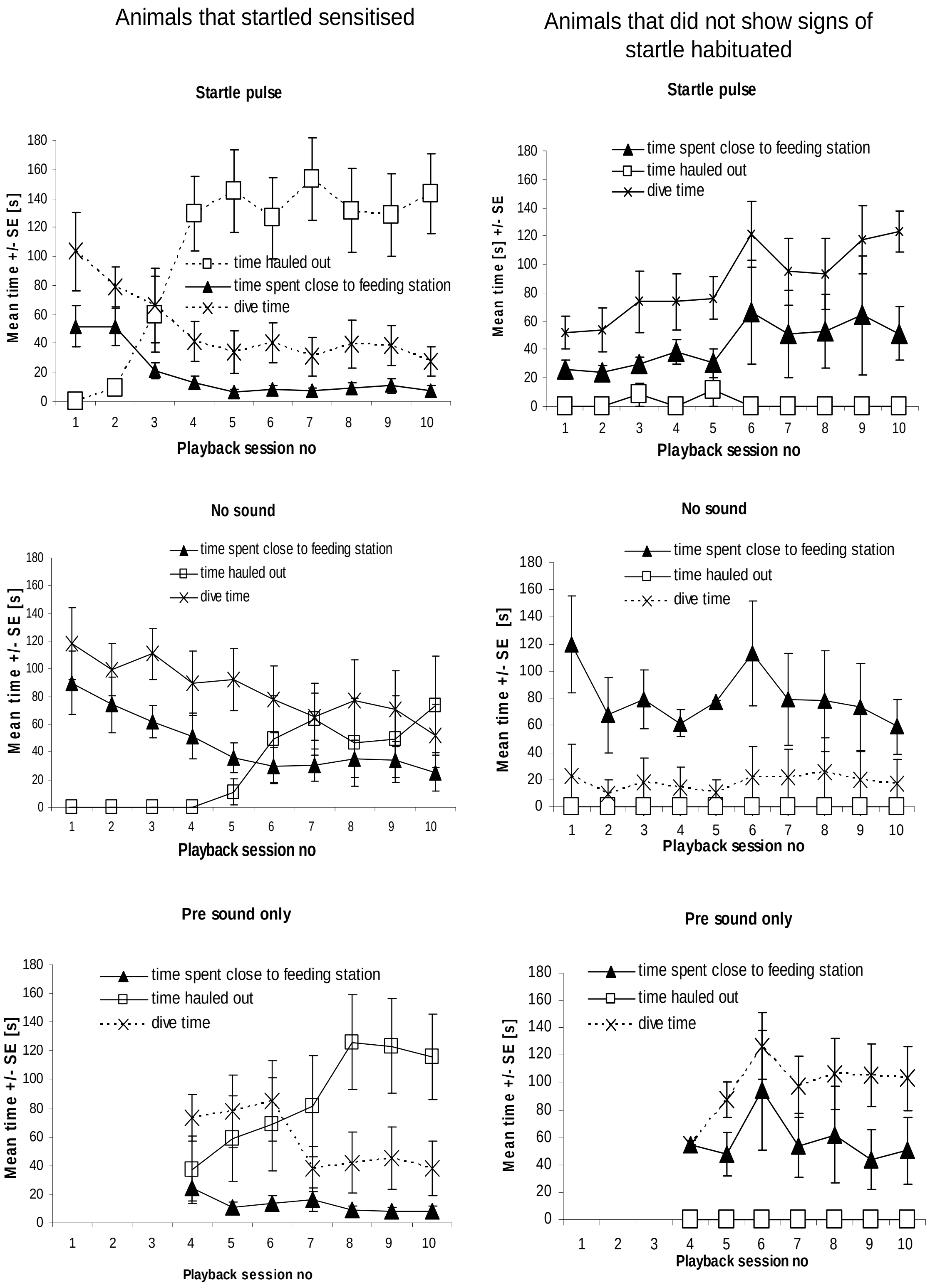


Figure 3: Response variables time spent close to feeding station, haulout time & dive time for animals that startled (left column, n=5) and animals that did not show signs of a startle response (right column, n=3). Animals that startled sensitised to the sound exposure as well as to the general experimental setup (e.g. see haulout behaviour during no sound control in the last playback sessions).

The GLM for “time spent close” in the animals that habituated explained only 32% of the variance with individual variation being the strongest factor, followed by treatment (GLM, $F_{17,\ 63}$=12.4, p<0. 0004, r^2_{adj}=0.32). The interaction between individual and treatment was also significant. Dive time was mostly influenced by individual variation and to a lesser extent by the sound treatment (see table 1, GLM, $F_{17,\ 63}$=7.0, p<0. 0001, r^2_{adj}=0.56). None of the animals that habituated ever hauled out during any of the experimental sessions.

Flight responses and interruption of foraging behaviour

The exposure to the noise was followed by fast and immediate flight responses in all animals that showed signs of startle reflex elicitation (see fig. 4a). Flight responses first occurred infrequently but from playback session 5 on exposure to the startle pulse was almost always followed by a flight response (see fig. 4a). A similar but delayed trend was seen for the “pre sound only” treatment. The likelihood of eliciting flight responses increased steeply in playback session 5 to 6 and the pre sound always caused a seal to flee in playback sessions 9 & 10. In contrast, flight responses only occurred infrequently in animals that did not show signs of startle and vanished altogether after a few exposures. Flight responses in seals that did not to startle also involved slower movement away from the feeding station and were never followed by haulout behaviour. In contrast, in animals that did startle, flight responses were always followed by a sudden, fast jump out of the pool and extended haulout behaviour in playback sessions 7-10. The likelihood of causing a flight response was modelled for animals that startled using logistic regressions of the type $y= a/(1+e^{(-(x-xo)/b})$ with a, b, and xo being parameters that were adjusted to fit the data., x being the playback session number and y the % of events during which a response occured The regression for exposure to the startle pulse was highly significant and explained 97% of the variance in the data set ($F_{2,7}$=140, p<0.0001, r^2=0.97). The regression for the pre-sound only treatment shows a similar but delayed trend indicating that classical conditioning had formed a link between the pre-sound and the startle pulse after several more pairings ($F_{2,7}$=53.0.0, p<0.0001, r^2=0.95).

Foraging behaviour was interrupted by sound exposure in both groups of seals in the beginning of the experiment. Interruption of foraging involved the animal pulling its head back from the cup and failing to retrieve the fish for the whole 3 min observation period. The likelihood of foraging behaviour being interrupted shows an inverse

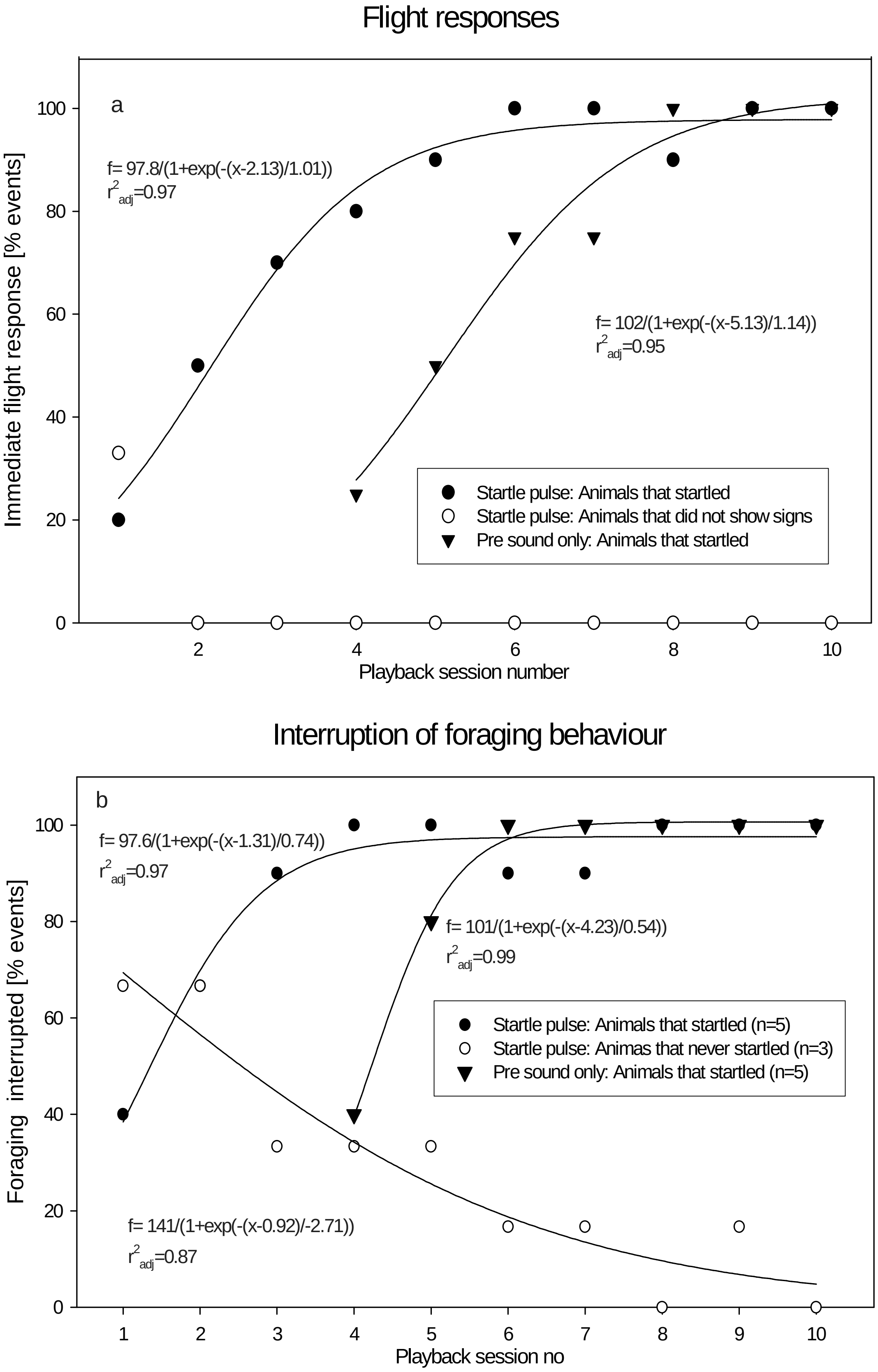


Figure 4: Percentage of startle pulse presenations after which the animals showed an immediate fast flight response (a) and (b) foraging behaviour was interrupted instantly (animal failed to retrieve the fish from the cup). Sigmoidal functions were fitted to the data points showing an increase in the probability of occurrence of flight responses and interruption of foraging behaviour in animals that startled. The opposite trend was observed for animals that did not show signs of startle.

pattern for the seals that showed signs of startle compared to those that did not. A sharp increase in the probability of foraging behaviour being interrupted was seen in animals that startled and foraging behaviour was always prevented in playback sessions 8-10 (see logistic regression in fig 2b; $F_{2,7}$=534, p<0.0001, r^2=0.99). The model for the “pre-sound only” treatment for the animals that startled showed a similar but delayed increase in the likelihood of interruption of foraging ($F_{2,7}$=30.0, p<0.0004, r^2=0.87). The logistic regression for animals that did not startle showed a continuous decrease in the likelihood of interruption of foraging behaviour ($F_{2,7}$=65.2, p<0.0001, r^2=0.93). The “pre-sound only” treatment did never interrupt foraging behaviour in animals that did not show signs of startle reflex elicitation.

Additional experiments on a subset of animals that sensitised : Stimulus generalisation and long-term effects of sound exposure

Three seals were tested on the occurrence of flight responses when a random selection of sounds from experiment 1 in chapter 3 was played. Two sounds were tested with each animal. Since these sounds had never been paired with the startle pulse and animals had previously habituated to these sounds (see experiment 1 of chapter 3) the test was meant to provide some preliminary information on stimulus generalisation with respect to conditioned aversive behaviour. Two of the three seal (one adult & one juvenile) responded instantly with a fast flight response while the third seal did not show an overtly high avoidance response.

Furthermore, two juveniles that had sensitised were tested again 3 months after the end of the original experiment. Due logistic limitations and time constraints this must be considered a preliminary test. First responses to exposure of the pre-sound alone were tested. None of the animals sowed a flight response or any other overtly high aversive behaviour. Then animals were tested in the normal setup for 4 consecutive playback sessions (as described earlier). Both animals seemed to acquire flight responses slightly faster: The first flight response had occurred in playback session 2 and 3 respectively in the original experiment. However, in the re-test after the 3 month break the first fast flight response occurred already in playback session 1 and 2 respectively. The development in all other responses variables and the general pattern of the sensitisation process was similar to the original experiment. In conclusion, after 3 months of no sound exposure behaviour was more or less back to normal but animals might sensitise slightly faster than in the original experiment if they had previous experience with startle sounds.

Experiment 2: Startle thresholds in grey seals

Methods

Playback and monitoring equipment were as in experiment 1. Threshold measurements were carried out after experiment 1 was finished. The main problem was that due to the strong aversiveness of the stimuli in the previous experiment it proved very difficult to attract animals to the feeding station. In animals that had sensitised in the previous experiment more than a day’s break and substantial food rewards were required to make the animal approach the feeding tube again (and in some cases the animal would still only approach for a short time). After an animal got accustomed again to wait in front of the feeding station experiments were carried out. The edge of the cup was lowered as a cue for the seal to position itself in front of the feeding station. If the animal was closer than 40cm either lying on the bottom of the pool or drifting motionless in front of the feeding station the playback started. This involved presentation of 1 kHz and 200ms long pure-tone pulses with rise-time of 5ms. These pulses ranged in intensity from 140-180 dB re 1µPa in increments of 5 dB. Since subtle responses like neck twitches could only be analysed appropriately by watching the videos from the underwater camera on a big screen, no classical staircase design was applied which would have required a decrease or increase of stimulus intensity depending on the observed response. Therefore, all nine intensity steps were presented in a pseudo-randomised order. Four playback blocks each containing all nine intensities in a different pseudorandomised order were carried out for each of the eight animals. A 1min interval separated each intensity step within a block. The time interval between the end of one block and the start of the following block was at least 1h. No more than 2 blocks were presented on any one day. If two blocks were measured on the same day a 12h recovery period was introduced before the third block was presented on the following day. The startle threshold was defined as the 75% response threshold. This means that an animal had to exhibit a neck twitch or a whole body startle in at least 3 out the 4 blocks at a defined received level and all higher received levels. Behaviour was coded from the videos as “neck twitch” or “whole body startle” if there was a clear shift (“jump” or “curving”) of the whole body in the video frame. The highest intensity stimulus was only presented twice in the two sessions furthest apart. This was done because the maximum sound pressure level was potentially getting close to the threshold for onset of temporary threshold shift (TTS) which has been determined to occur at sound exposure levels

(SEL) of 183 dB re 1µPa-s dB in a harbour seal (Kastak et al. 2005). Given that the 180 dB re 1µPa stimulus tested in my study had a sound exposure level of only 173 1µPa^{2}s^{-1} (due to its short duration) and the hearing thresholds at the frequencies tested by Kastak et al 2005 were lower, my stimulus can be expected to have had no impact on hearing. However, higher intensities were not tested in this study to account for the uncertainty in the limited data available on onset of minor TTS in pinnipeds. In addition, the loudspeaker started to distort the signals at levels higher than 180 dB re 1µPa.

The 200ms long 1 kHz pure tone signals were synthesised in Cool Edit pro in 5 dB amplitude steps. The correct received levels for the threshold measurements were obtained through a series of sound field measurements in the pool. First, the power amplifier was roughly adjusted to produce the expected output. Then in a series of calibration trials prior to the experiment the output of the power amplifier was adjusted so that the 9 intensities would coincide with received level ranging from 140-180 dB re 1µPa. At lower amplitudes this also required an adjustment of the signal in the digital domain. Received levels were then measured at 8 different positions where seals typically positioned their head when stationing in the experiment. Each position was measured several times on different days and values were then averaged over all eight positions. The variation of received levels between the measured 8 positions was on average +/- 3 dB. If the pool had to be emptied and equipment had to be removed between tests of different individuals a new calibration was carried out. Slight changes in orientation of the transducer had no apparent systematic effect on measured received levels. No changes in the output of the transducer were found over the course of the experiment from 2006 to 2007.

Results

The startle thresholds are given in table 2. Startle responses occurred at received levels down to 155 dB re 1µPa in two animals (see table 2). Startle thresholds defined as 75% response threshold to 1 kHz pulse were found to be between 160-165 dB re 1 µPa for all animals that sensitized in the previous experiment. Taking the hearing threshold from the composite audiogram (see appendix 1) into account, this would reflect a sensation level of 80-85 dB. However, no startle threshold could be determined for the three animals that habituated in experiment 1. All three animals showed no apparent skeletal muscle contraction, eyelid closure or any other signs that would be indicative for the startle reflex. This includes the two exposures to the loudest stimulus which had a received level of 180 dB re 1µPa.

Test order, species (HS: Harbour seal, GS: Grey seal	Age when tested (unknown for adults)	Startle threshold (dB re 1 µPa) 75% response threshold	Lowest RL to which startle occurred (dB re 1µPa)	Behaviour in experiment 1
1 HS male	adult	>180	-	habituated
2.GS female	7 month	160	155	sensitised
3.GS female	adult	>180	-	habituated
4.GS female	adult	160	155	sensitised
5.GS male	11-12month	165	160	sensitised
6.GS female	7 month	160	155	sensitised
7.GS female	7-8 month	165	160	sensitised
8.GS female	adult	>180	-	habituated

Table 2: Startle thresholds and life history information for the 8 seals tested in experiment 1 and 2. No startle threshold could be determined for the three animals that habituated in experiment 1 since they never showed any sign of startle.

Experiment 3: Field trials investigating larger scale flight responses

Methods

The methods were the same as in experiment 2 in chapter 3. Startle sounds were part of the playback sequences described in experiment 2. The startle stimulus was the same as in experiment 1 except for that the pre sound was not played. The source level was 180 dB re 1µPa @ 1 m. 12 startle pulses (200ms long) were presented in an irregular sequence over the 5min playback period. The pulse interval ranged from 10-40seconds. The startle pulse was the same as in experiment 1. The sound field measurements were also the same as in experiments 2 in chapter 3.

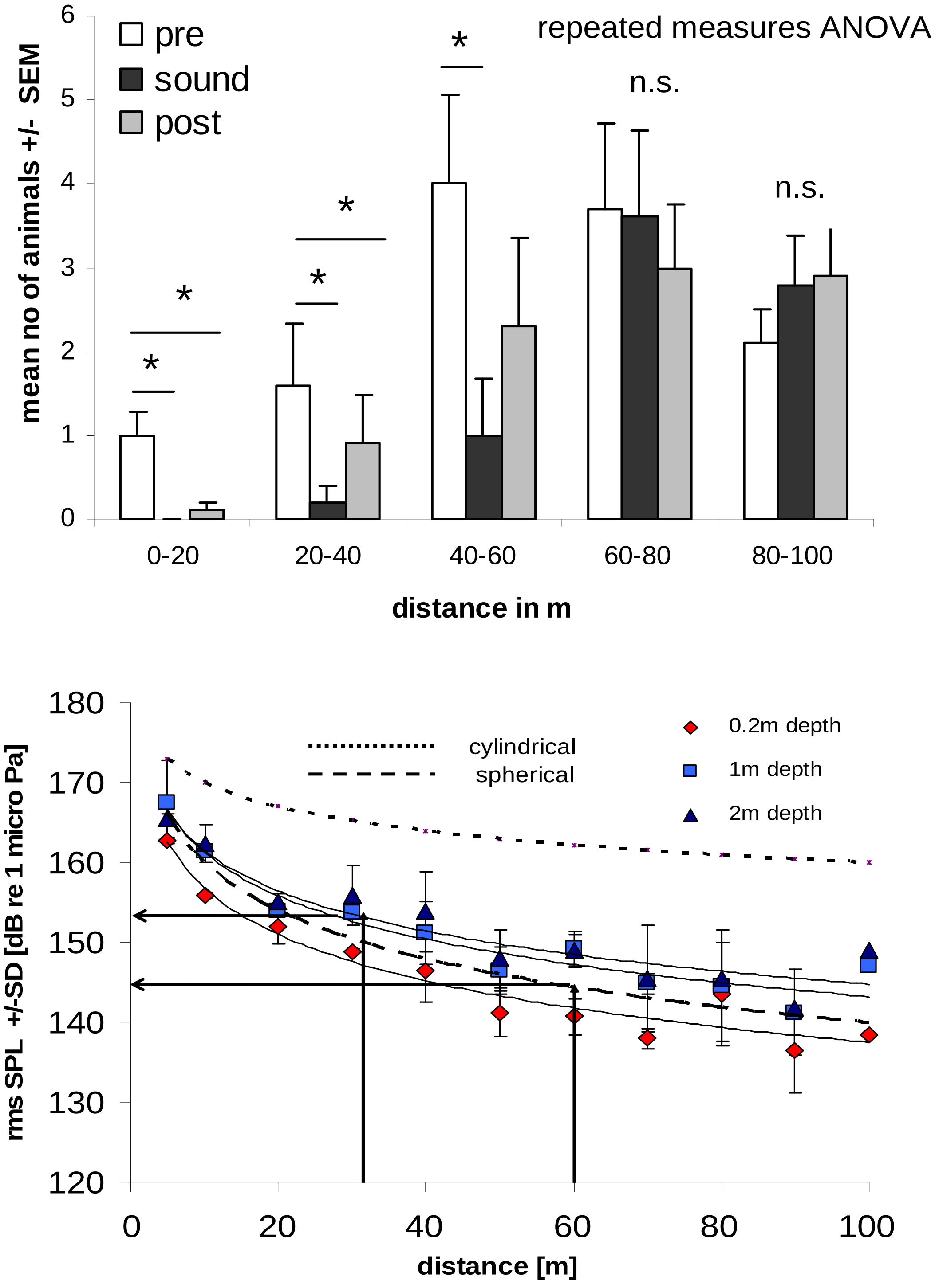


Figure 5: Mean number of seals in each distance bin during the pre sound, sound exposure and post observation period. The lower panel shows the average measured received levels for the two profiles at three different depth. Arrows indicate received levels at the edge of the deterrence range (60m, zone within which significantly less seals were counted) and at the end of the zone where no seals were seen at all during sound exposure (26m). The curves “cylindrical” and “spherical” show theoretical spreading loss without taking absorption into account.

Results

Figure 5 shows the mean number of animals in each 20m distance bin during pre-sound, sound exposure and post observation period. Repeated measures ANOVA showed that sound exposure caused a significant decrease of seal numbers in the three distance bins from 0-20m, 20-40m and 40-60m ($p<0.05$), The closest ever observed approach of a seal during sound exposure was 26m and very few animals were seen at distances of less than 40m. Furthermore, fewer seals were seen during the 5 min post sound exposure period compared to the pre-observation period for the distance bins 0-20m and 20-40m (adjustment for multiple pos-hoc tests according to Sidak (1967): pre versus sound and pre versus post). This shows that deterrence effects lasted longer than 5min post exposure. Corresponding received levels at the different distances can be read from figure 5. Animals that had their head (and ears) half submerged at distances of 30-40m of exhibited sudden “crash dives” in response to the first startle pulse. This means that animal vanished suddenly underwater producing a big splash and usually no animals were seen at the surface for the following 2-3 min. This was observed in 7 out of the 10 playbacks. Then, a similar amount of animals started surfacing at higher distances in parts outside the observation range. In one case a juvenile and in another one an adult were seen at about 8m and 12m from the boat right under the water surface when the playback started. Both animals were seen to be darting off underwater at very high speed.

Finally the number of animals within the whole observation area (up to 100m from sound source) was compared between the “pre-sound” (mean=11.4, SE=1.47) and “sound” treatment (mean=7.4, SE=1.94). The mean number of seals within the whole observation area (<100m) differed significantly between pre-sound observation period and sound exposure (Friedman test, F=6.4, p=0.11, df=9).

Discussion

Behavioural follow-up responses and startle reflex elicitation: What determines sensitisation/habituation of avoidance behaviour?

In experiment 1 the majority (n=5) of the eight animals sensitised to the sound with responses being strongest in the last three playback sessions. These animals developed immediate flight responses, spent most time hauled out on land by the end of the experiment and became extremely reluctant to approach the feeding station. This result is striking given that 3 of the 5 animals that sensitised to the startle pulses had previously been tested in experiment 1 described in chapter 3 where they had habituated very quickly to all sounds tested. While habituation to sounds has been commonly observed in marine mammals (Teilmann et al., 2006; Deecke et al., 2002) there one documented case of a single individual sensitising to repeated sound exposure (kastak & Schusterman, 1996). To my knowledge this is the first example of several animals sensitising to anthropogenic noise for any marine mammal species in a context where food motivation was high.

The results for the 5 animals that showed sensitization do however stand in contrast to the three animals that habituated posing the question what factors account for the difference in behaviour between the two groups. Animals of both groups showed similar baseline behaviour before the experiment except for one seal in the group that habituated which was scared by the presence of a human around the pool. Four out of the five animals that sensitised to the sound were juveniles/sub-adults and one was a mature adult female while all three animals that habituated were adults. None of the animals were deaf as all subjects responded to sound initially and had been tested in a previous experiment where they showed clear movement responses to conspecific calls at received levels as low as 125 dB re 1µPa (see chapter 4; experiment 1). The only consistent observable difference to the playback of the first startle sound between the two groups was that all animals that sensitised showed a clear and sudden neck or body muscle contraction while animals that habituated did not. This means that the startle reflex was elicited in all animals that sensitised while there was no clear evidence for startle in the animals that habituated.

Startle responses can have very small amplitudes. It is known that stimuli which only just exceed the startle threshold will elicit very small muscular responses (Pilz et al., 1987) that are maybe less likely to be detected by visual observation. Startle response magnitude is also influenced by emotional state of an animal with conditioned fear increasing responses (Brown et al., 1951) and conditioned pleasure leading to a reduction (Schmid et al., 1995). In humans, the startle response magnitude decreases quickly during habituation with only an eye-blink response remaining after a few exposures which can be quantified by measuring sensitive electro-myographic methods (see Blumenthal et al., 2005 for a review). However, exposure to the first pulse is known to cause an overtly high muscle contraction in rodents (Moyer, 1963) which is less likely to be missed visually. Therefore, it seems unlikely that signs of startle were missed in playback sessions of experiment 1 and 2. This is particularly true for the second experiment in which animals were only exposed to a test tone when they were seen floating motionless or with little movement in front of the video camera which would have increased the likelihood of detecting muscle activity above baseline.

One logical explanation for the lack of a startle response in some individuals may be that these animals had a higher startle threshold and the received levels tested in this study were not high enough to elicit the reflex. This could be the result of elevated hearing thresholds due to age related hearing loss (*presbycusis*) or noise exposure in the wild. There is good evidence from the literature supporting this notion: First, the startle threshold has been shown to be more or less parallel to the hearing threshold in rodents (Pilz et al., 1987). Furthermore, the fact that phenomena like pre-pulse inhibition of the startle reflex can be used for audiometry (Young & Fechter, 1983) highlights the role of the hearing threshold with respect to the startle reflex. In addition, mice showed a complete lack of startle caused by hand claps and short metallic sounds if their hearing threshold was higher than 76 dB re 20 µPa (Jero et al., 2001). However, most importantly a clear relationship between startle and hearing thresholds was found in a study on age-related hearing loss in different mice strains (Ouagazzal et al., 2006). The authors measured startle behaviour and hearing thresholds (using ABR) in mice strains that develop progressive age-related hearing loss (CB 57BL & 129) and a hybrid strain that developed little hearing loss with age. Initially, at a young age (e.g. 28 weeks) strain 129 and the hybrid strain had comparable startle thresholds at 80-85 dB re 20 µPa while CB57 had slightly higher startle thresholds (95dB re 20 µPa). However, at high ages strain CB57 which had developed the most pronounced hearing loss failed to show any signs of startle even to the loudest stimulus tested, similar to the results in my study. Strain 129 with less pronounced but still substantial hearing loss did show a startle reflex but only in some cases and only in response to the loudest sound pressure levels tested (120 dB re 20 µPa). In contrast, the hybrid strain with little hearing loss had startle thresholds as low as 100 dB re 20 µPa. This clearly shows that louder stimuli are needed to cause a startle response in animals that have elevated hearing thresholds. Most strikingly, the amount of hearing loss in the 129 strain (40 dB) and the hybrid strain (10-15 dB at lower frequencies) closely matched the elevation of the startle threshold. Strain 129 showed an elevation of the startle threshold by about 40 dB (from 80dB to at least 120dB re 20 µPa) while the hybrid strain showed an elevation by 15 dB (from 85dB to 100dB re 20 µPa). Startle thresholds for the majority of the tested seals were found to be 160 dB re 1µPa while the three animals that never startled must have had thresholds higher than 180 dB re 1µPa. To explain the lack of a startle response in the three subjects their hearing threshold would have to be elevated by about 20 dB. Although normal hearing loss as a result of age (presbycusis) is more pronounced at higher frequencies, threshold shifts of 10-20 dB are common in human subjects aged over 60 years even at mid or lower frequencies (Hinchcliffe, 1959). Given that the animals tested in my experiment were wild caught from the North Sea (an area with high industrial noise) inter-individual variation in hearing can also be expected to be high as a result of noise exposure. Inter-individual variation of bottlenose dolphin hearing thresholds and presbycusis measured through auditory evoked potentials (AEP) is well documented for large captive populations and was found to be at least 20-30 dB across subjects at mid-frequencies (Houser et al., 2008; Houser & Finneran, 2006).

In conclusion, in the light of the mentioned literature it is quite possible that seals that did not show signs of a startle reflex may have had slightly compromised auditory functions, namely 20 dB higher hearing thresholds. This would in turn lead to a higher startle threshold and the tested stimuli might have therefore not been loud enough to elicit the startle reflex. Since animals that did not show any signs of the startle reflex habituated, it seems likely that startle reflex elicitation plays some role for the development of flight responses, interruption of foraging behaviour and sensitisation to sound.

Startle thresholds and previous observations of startle responses in marine mammals

The startle threshold in rats and mice ranges from 80 to 95 dB above the hearing threshold depending on background noise and strains tested (Fleshler, 1965; Pilz et al., 1987; Ouagazzal et al., 2006). Startle thresholds measured for the 5 seals that sensitised in experiment 1 were between 160-165 dB 1µPa. When these results are translated into sensation levels using published audiograms for harbour seals (see results) startle thresholds for seals would lie between 80-85 dB. This means that stimuli that exceed the hearing threshold by a similar amount are capable of eliciting the reflex in terrestrial mammals compared to pinnipeds. Therefore, pinnipeds do not seem to have special neuronal adaptations to aquatic hearing that may have altered their startle reflex. The startle thresholds measured in experiment 2 are also in line with behavioural responses observed in field trials (experiment 3). The measured received level at the edge of the area where no seals were observed during sound exposure (<26m) was approximately 155 dB re 1µPa (fig 5). This value exactly matches the lowest received level that has been found to cause startle in two of the animals in the threshold measurement experiment (exp 2). However, received levels in the area within which significantly less seals but still some seals were counted (60m) were lower (145-150 dB 1µPa). This might reflect the fact that movement or flight responses of seals extended beyond the zone where the stimulus was loud enough to elicit startle. Alternatively, it could mean that the startle pulse still had some moderate deterrence effect at lower received levels.

Previous evidence for startle in seals is sparse. High source level acoustic deterrence devices (Mate & Harvey, 1987) or explosives like “seal bombs” used to scare seals from fishing boats (Cassano, 1990) may be able to cause such responses but generally no controlled observations are available in these situations. Kastelein et al. (2006) tested the deterrence effects of short tone pulses on harbour seals and reported no signs of an “initial startle responses” but correctly admitted that such a response might have been easily missed below the water surface. Given the more sensitive hearing at the frequencies tested by Kastelein et *al*.(2006c) the source level of 149-161 dB may have just been loud enough to cause startle in an animal very close to the loudspeaker but probably not at distances of more than 3-5m. Kastelein et *al*. 2006 did not find evidence for habituation and sounds were effective in deterring seals for one month. Another interesting observation comes from an experiment in which cod were exposed to seals or predator like shapes. They were found to produce short (<1ms) clicks with almost zero rise-time at source levels of up to 153 dB re 1µPa (Vester et al., 2004). The seals seemed to interrupt approaches of cod when the fish produced loud clicks but capture attempts were never interrupted in case of non-clicking salmon. It may be that these clicks did under certain circumstances exceed the startle threshold and therefore caused the seal to abort a catch attempt.

The term “startle” has also occasionally been used to describe behavioural and physiological responses in cetaceans (Teilmann et al., 2006; Southall et al., 2008). Unfortunately, among marine mammal researchers the term seems to be rarely used in its widely accepted physiological meaning (see Koch, 1999) making it difficult to evaluate the literature. For example, Teilman et al. (2006) tested responses of porpoises to acoustic alarms and defined “startle” as a change in the average heart rate. They found cardiac and avoidance responses to wane rapidly. Their definition of startle is problematic since experiments on mice clearly showed that although startle leads to an increase in heart rate a specific startle-related cardiac response does not exist and cardiac responses can occur in situation where no startle is present (Tovote et al., 2005). Therefore, a cardiac response cannot count as evidence for a startle response but one needs to look for signs of the activation of the motor-neurons instead (muscle contraction or sudden eyelid close). Teilman et al. (2006) also reported an acceleration of swimming speed in response to a broadband noise pulse with a source level 132 dB re 1µPa on a single occasion. Consecutive louder signals did however not cause a response. The noise sound in Teilmann’s study (in contrast to other test sounds) seemed to be the most suitable for elicitation of a startle response due to its short rise time and high bandwidth (read from the sonogram). The hearing threshold of a porpoise is 32 dB re 1µPa at 100 kHz (Kastelein et al., 2002) meaning that if the animal was 1m from the transducer the sensation level was about 100 dB. Depending on the distance of the animal from the transducer this signal might have just exceeded the startle threshold which would in turn suggest that startle thresholds in cetaceans might be similar to seals. One underlying reason why behavioural avoidance responses in porpoises were subject to habituation may have been that stimuli did not exceed the startle threshold in the majority of the sessions either as a result of received levels being not high enough or because rise times in some of the stimuli were too long. In a similar experiment Kastelein et al. (2001) tested acoustic alarms in a porpoise and found that the sound of the highest source level (163 dB re 1µPa @ 1mv) which also seemed to have the shortest rise-time (see fig 3 in Kastelein 2001; this refers to the sound XP-10) caused the strongest deterrence effect. Although the authors conclude that little of the behaviour was due to ”startling”, the stimulus could have been loud enough to cause a startle response and its high efficiency might have been a result of reflex elicitation.

In conclusion, startle responses may have been responsible for some avoidance behaviour observed in marine mammals, however, none of the studies measured the reflex e.g. by quantifying the activation of the moto-neurons by monitoring muscle contractions. Therefore it cannot be said for sure what factors might have lead to the observed follow-up behaviour reported in other studies.

Biological function of startle responses: Increasing the propensity of an animal for flight ?

In spite of the vast literature on the neurobiology (Koch, 1999) and clinical applications of the startle reflex (Meinck, 2006), no systematic study has targeted the question of the biological function of the mammalian startle response from a behavioural perspective. In fish, a seemingly homologous reflex is mediated by stimulation of Mauthner neurons that receive input from the lateral line and the ear leading to a directional small-scale “C-start” in the order of tens of centimetres (e.g. Blaxter et al., 1981). The C-start does, however, not seem to cause larger scale movement responses away from a sound source (Wardle et al., 2001). In some insect species, intense ultrasonic sound pulses elicit startle responses that initiate escape responses away from the sound source (Hoy et al., 1989). Traditionally, the evolutionary origin and function of the mammalian startle response has been seen in interruption of ongoing behavioural patterns (Landis & Hunt, 1939) or in a protection against injury from a predator and in a preparation of a flight response. Yeomans et *al*. (2002) argued that data on cross-modal integration of information in the startle pathway point towards a function in protection against sudden mechanical hind-head blows, a stimulus which would activate the auditory, vestibular and tactile pathways simultaneously. Although the startle reflex does not directly cause an animal to flee, it initiates the activation of the sympathetic nervous system leading to an increase in heart rate (Korn & Moyer, 1966) as well as an increase in coordinated muscle activity (Pilz & Schnitzler, 1996). Together with the shortening of the response latency over several exposures this suggests a function in increasing the animal’s propensity for flight e.g. in a predator avoidance scenario (Pilz & Schnitzler, 1996). Although predators will generally aim to produce as little noise as possible an approaching carnivore in a woodland habitat might accidentally break a twig on the ground, and therefore produce a short click that would elicit startle. The role of the acoustic startle response in predator avoidance is also hinted at by the fact that response magnitude can be increased by temporarily decreasing illumination as would be the result of the shadow cast by a moving predator (Ison et al., 1991). The strong correlation between startle elicitation and the occurrence of flight responses in the tested seals points towards a function in preparing quick flight responses. Despite the fact that flight responses are under voluntary control of the animal (in contrast to the muscle contraction) they were exhibited in a surprisingly stereotyped manner after few sound exposures.

It may be that the startle reflex causes physiological changes in an animal that are associated with fear and therefore lead to a consecutive flight response. While a wealth of data is available on startle reflex magnitude as a measure of fear (Brown et al., 1951) almost no reliable information is available on the role of the startle reflex itself as a potential unconditioned stimulus (UCS) inducing fear (see Watson & Rayner, 1920 for a famous but unreliable and much criticised account of startling noise causing unconditioned fear in a human subject). Most animal experiments on avoidance learning and conditioned fear use electric shocks as unconditioned stimuli (Miller, 1941). It would therefore be interesting to test whether loud acoustic stimulation alone is sufficient to induce fear. The fact that the startle pulse acted as an efficient unconditioned stimulus and seals behaved similar to rats in “fear conditioning paradigms” shows that this is a likely scenario (see following section for detailed discussion of fear conditioning).

Bolles (1970) argued that some avoidance behaviours cannot be learnt in laboratory settings while others are readily acquired. He argued further that therefore much of what is seen in avoidance learning contingency experiments in the laboratory is primarily a result of genetically determined species-specific defence reactions (SSDR) and suppressed alternative behaviour. Avoidance strategies in more naturalistic settings depend on a variety of more complex factors e.g. the distance to a predator might decide if freezing or flight is an appropriate avoidance response (Eilam, 2005). For terrestrial mammals in complex habitats (like a woodland) a different behavioural reactions (e.g. freezing) might constitute a successful avoidance tactic in many situations. In contrast a seal or another small marine mammal like a harbour porpoise foraging in the open sea alone or in small groups has probably little chance of hiding or fighting a predator which would mean that fast, long distance flight is the only chance for escape. The field trials showed (experiment 3)flight responses might be exhibited over medium distances: the analysis by distance bins suggested that seal numbers dropped significantly during sound exposure at distances of less than 60m and that the overall number of seals was lower in the whole observation area up to 100m. Also, seal numbers were lower during the 5min post sound exposure in the distance bins closer than 40m. Given that the habitat where playbacks were carried out was spatially quite constrained this can (on a relative level) be considered a “larger-scale” movement response. Most aquatic top predators will hunt in silence (e.g. sharks), however, if echolocation clicks of killer whales could elicit acoustic startle then the reflex would be highly adavantegous for seals. In case of silent predators (e.g. sharks) a well developed startle pathway might still be relevant since tactile startle caused by the shockwave of a large approaching object could increase the chance of a last minute escape.

Fear conditioning

The behaviour observed in response to the pre-sound after a few pairings with the startle pulse can be explained in the context of classical avoidance learning and fear conditioning. In Pavlovian fear conditioning (e.g. Miller, 1941; Mowrer, 1947) an unconditioned stimulus (UCS, startle pulse) which elicits an unconditioned avoidance response (UCR, e.g. flight; jump out of the pool) is paired with a conditioned stimulus (CS, pre-sound) which carries initially no aversive properties. After a few pairings, the CS can then elicit a similarly strong avoidance response as the UCS and conditioned avoidance behaviour can be resistant to extinction for some time. The behaviour of seals is therefore similar to results from avoidance learning experiments in rats where animals were trained to show an unconditioned avoidance response in response to a conditioned external stimulus (e.g. Theios, 1963). In my experiments, the pre-sound reached a 75 % probability of eliciting a flight response in the 6th playback while the startle pulse reached the same criterion in the third playback session (see fig 4 a). Due to the responsive mode nature of the playbacks this reflects a range from 7-9 pairings for the different individuals. The difference between the startle pulse and the pre-sound alone reaching the 75% response criterion was only 3-4 pairings. The amount of pairings needed to elicit a conditioned avoidance response strongly depends on whether the UCR that is to be put under stimulus control is similar to naturally occurring avoidance behaviour (a species-specific defence reaction according to Bolles, 1970). Since stereotypic flight responses and “jump out of the pool behaviour” were acquired relatively quickly such behaviour might constitute a SSDR. Although seals on land generally flee for the water when being disturbed, certain predator avoidance scenarios e.g. predation by killer whales lead to retreat into shallow water and haulout (Deecke et al., 2002). Finally, the fact that the startle pulse acted as a surprisingly effective UCS suggests that it might cause fear and therefore increases the likelihood for flight responses.

Avoidance learning and approach-avoidance conflict

Figure 6: Adult grey seal observing the feeding station while being hauled out after the sensitisation process in experiment 1.

The movement and haulout behaviour of the seals in experiment 1 was comparable to avoidance learning in laboratory studies on rodents where animals had access to a safe place for retreat (Crawford & Masterson, 1978). Surprisingly, flight responses were at first less likely to occur but increased over time. However, one needs to keep in mind that the experimental setup in experiment 1 resembles a typical approach-avoidance conflict scenario (e.g. Brown, 1948; Hoffman & Fleshler, 1963a). In approach-avoidance conflict experiments animals are trained to show a conditioned approach response which is then in later trials put into conflict with an avoidance behaviour. This is achieved by pairing any conditioned approach with presentation of an aversive stimulus (e.g. electric shock) which then in turn makes the animal hesitate to approach. Similarly, in experiment 1 the seal had to choose between a) approaching the food source on cue (edge of cup becoming visible), obtaining a food reward but being exposed to the startle pulse or b) leaving the pool (avoidance) without food reward but avoiding the startle pulse. Within the first two or three playbacks an approach-avoidance conflict was often visible in the most classical sense. The seal was behaving as if being pulled between two antagonistic forces moving forward towards the feeding station and suddenly retreating again in quick succession. The general outcome of this conflict is also visible in figure 4 which shows decreasing foraging success and increasing flight responses over consecutive trials. In rats, the tendency to approach a feared goal (e.g. food source that elicits electric shocks) is higher when an animal is further away from it meaning that at close distances the strength for avoidance exceeds the “drive” to approach (Brown, 1948). Similarly, avoidance behaviour won over approach at close distances in experiment 1. Seals would often wait hauled out only having the eyes submerged in the water until the cup became visible (figure 6). Then the seal would enter the pool for a fast approach, immediately aborting the approach on hearing any disturbing noise (pre sound or rattling of sound from feeding station). In lab experiments, hungry (food deprived) rats have been shown to be more willing to approach in conflict situations (Brown et al., 1966). Also, previous experience with a food source seemed to be important as rats that where “overtrained” for approach showed higher signs of conflict and emotionality (Kempe & Brown, 1956). The 5 seals that sensitised in experiment 1 were food deprived for at least 12h prior to the first playback which made them interested in the food source but does probably not reflect a very high level of motivation. The tested seals could be considered an “overtrained” group since they had all been tested in previous experiments where they learnt to associate the feeding apparatus and the appearance of the aluminium cup with food. It is also interesting to note that prior to the experiment only a slight noise from the cup being lowered through the feeding tube caused an immediate approach response. In contrast by the end of the experiment any rattling sound from the tube caused the animals that sensitised to retreat.

Startle eliciting sound pulses as an acoustic deterrent

The startle reflex appears to hold great potential for use in ADDs. While the initial reaction was comparable to that found in response to the new sounds and the commercial ADD sounds tested in chapter 3 (experiment1), the majority (n=5) of the animals sensitised to the sound with responses being strongest in the last three playback sessions. It is also important to note that exposure to the startle pulse led to a general reluctance to approach the food source by the end of the experiments even during no sound control sessions. While previous lab experiments on marine mammals have commonly found habituation (Teilmann et al., 2006) or short-term stable responses (Kastelein et al., 2006) to my knowledge sensitisation has not been previously demonstrated in a context where food motivation was involved. The data also showed that it is possible to use conditioning to elicit flight responses by pairing a non-aversive stimulus (pre-sound) with an unconditioned stimulus (startle sound). This could be applied to greatly reduce noise pollution around fish farms because the loud, unconditioned stimulus (the startle pulse) would not have to be presented in every playback. In addition, target-specificity could be increased by making use of species differences in hearing thresholds which can be expected to underlie differences in startle thresholds.

Marine mammal noise exposure criteria

The presented data have implications for marine mammal conservation as they may suggest a reconsideration of recently published noise exposure criteria (Southall et al., 2008). A severity ranking scale for aversive responses to noise was published as part of the currently most comprehensive attempt to define noise exposure criteria for marine mammals (Southall et al., 2008). According to this ordinal scale the behaviour observed in the majority of the seals would have to be considered to fall in the second most severe category (score 8 out of 9). The authors define this category (score 8) as "avoidance of or sensitization to experimental situation or retreat to refuge area (> duration of experiment)". The sensitisation effects observed in this study have been discussed previously and the extensive haulout behaviour during the "no sound control treatment" should clearly count as "retreat to refuge area". There is also some evidence for the last condition that avoidance effects lasted longer than the duration of the experiment. Playback sessions were separated by at least 15 min and sometimes even by more than 10-12h since experiments were conducted over three days. The fact that animals showed haulout behaviour and aversive responses during the first "no sound control" on each playback day after the start of the playback indicates that sensitisation to the experimental situation lasted much longer than a playback session. This is also reflected by the lack of any significant decrease in the curves in figures 2, 3 and 4 over the course of the experiment (for the animals that startled). Since the 2 seals that were tested again after 3 months acquired flight responses more quickly than in the initial tests could also be considered weak evidence for longer lasting effects. If criteria for experiments on wild animals were applied (given that the animals were wild captured) then "flight responses "and "panic" would even justify a classification in the most severe category. "Panic" was observed in the sense that if seals entered the pool by the end of the experiment they would swim very erratically, quickly approaching and retreating in response to any noise in the pool. Given that some animals exhibited a flight response and interrupted foraging even in response to the first stimulus Southall et al's. (2008) statement that the "startle response to a brief, transient event is unlikely to persist long enough to constitute significant disturbance" should be looked at with caution (page 413).

The marine mammal noise exposure criteria published by Southall et *al.* (2008) suggest that exposure to a single pulse up to a sound exposure level of 171 dB 1µPa^2 -s or a peak sound pressure level of 212 dB re 1µPa would be acceptable for pinnipeds within the most sensitive hearing range. This criterion was, however, not based on behavioural data categorized by the severity scale but on data for the onset of temporary threshold shift, a physiological measure. Southall et al. 2008 argue that given the lack of behavioural data the occurrence of TTS can be used as an indication of behavioural disruption since it would also have an influence on behaviour. Although this is most likely true it does not mean that strong behavioural avoidance responses and sensitisation to sound cannot occur at much lower levels below the onset TTS. Some aspects of my experiments constitute a single pulse scenario e.g. if one only considers the responses to the very first startle pulse a seal was exposed to. In the field trials the first pulse caused "crash dives" making all animals "vanish"; diving animals were seen to dart off underwater at high speed. In the captive experiment the first startle pulse interrupted foraging at least in some seals (40% of the seals that startled). Southall et al. 2008 also suggest cautiously (based on the scarce literature) that exposure to "multiple pulses" in the range of 150 to 180 dB re µPa (rms) has only limited potential to cause avoidance in seals. My data shows that sensitisation to sound and even responses to single pulse with a short rise-time are more likely to be associated with the startle reflex threshold rather than the onset of TTS. Since the startle reflex threshold is much lower a re-evaluation of the noise exposure criteria is needed. The pulses tested in experiment 1 had a sound exposure level of 167 dB re 1Pa^2-s which is already lower than the criterion suggested for single pulses in the noise exposure criteria. However, since experiment 1 did not estimate the minimum SEL that causes extreme flight responses the data from experiment 2 (startle thresholds) should be used. I suggest that the noise exposure criteria for single and multiple pulses at frequencies of about 1 kHz should be set to the sound pressure level of the startle thresholds which is an rms SPL of 160 dB re 1µPa or a peak SPL of 164-165 dB 1µPa (the difference measured between rms and peak SPL in my test signal). An even better procedure would be to use a sensation level of 80-85 dB as a criterion and calculate the acceptable sound pressure level for each species and frequency band of the noise separately using composite audiograms. Although these lower values might cause serious problems for noise producers one needs to acknowledge that these criteria are based on observed extreme behavioural responses under controlled conditions. I also hypothesise that cetaceans would show similar responses and therefore noise exposure criteria for cetaceans should be replaced by thresholds for startle reflex elicitation. Although this hypothesis requires verification by experimental data one needs to consider that my study provides evidence for the relevance of startle for aversive responses and the startle reflex seems to be similar among mammalian taxa. Also, when choosing physiological measures one should give priority to those that are well understood on a theoretical level. While the actual behavioural relevance of temporary threshold shift is poorly understood, the neuronal circuits mediating startle-related motor behaviour have been described in detail (Koch, 1999). If similar sensation levels cause startle in odontocetes compared to pinnipeds (which is purely speculative) then startle threshold of at 16 kHz porpoise would be as low as approx 120 dB re 1 µPa (hearing threshold 44 dB re 1 microPa according to 1970).

Previous data on odontocete behavioural responses to pulsed sound (Schlundt et al., 2000; Finneran et al., 2000) conducted in captivity cannot be used to assess behavioural follow-up responses related to startle. This is because animals had been trained for years to respond to a variety of different sound stimuli and ignore others. More problematic, animals used in TTS experiments have been trained to specifically tolerate noise exposure, a procedure which is bound to alter behavioural follow up responses after elicitation of the startle reflex. Experiments should be carried out with animals from wild populations possibly simulating food motivation but not providing specific training to tolerate sound exposure. I also suggest that the startle reflex could be involved in extreme events like beaked whale mass strandings, a hypothesis that will be formulated in the general discussion (chapter 7).

Conclusions and summary

The presented data showed that the startle reflex is present in seals and seems to follow similar principles as in terrestrial animals. The startle reflex was found to be tightly associated with the occurrence of flight responses. In animals in which the sound stimulus was loud enough to elicit the reflex, aversive responses became stronger over time and animals sensitised to sound in spite of food motivation being involved. These animals showed a classical approach-avoidance conflict in the first few trials until avoidance responses became so strong that foraging behaviour was always interrupted and animals fled from the pool. In the three animals that did not show signs of a startle response, aversive responses were low and the animals habituated to the situation. This suggests that startle reflex elicitation is an important factor for the occurrence of flight responses and sensitisation to non-biological pulsed noise. It is suggested that repeated exposure to startle-eliciting noises leads to changes in the physiological state of the animal which are associated with fear and lead in turn to sensitisation to the sound. Pairing of an unconditioned weaker pre-sound with the startle pulse revealed that the startle pulse acts as an unconditioned aversive stimulus with just a few pairings of CS and UCS being sufficient to establish fear conditioning (meaning the originally non-aversive pre-sound can cause the same response as the startle pulse).

The startle threshold for the seals that sensitised was between 160 and 165 dB re 1µPa at 1 kHz for the 5 animals that startle in experiment 1. However, even the loudest stimulus did not seem able to elicit any obvious response in the three seal that habituated. When startle thresholds for the majority of the seals are expressed as sensation levels (80-85 dB) it becomes obvious that similarly loud sound stimuli seem to be able to elicit the reflex in pinnipeds compared to terrestrial mammals. The results from experiment 1 and 2 can might be explicable by assuming inter-individual variation in hearing thresholds. This assumption is supported by data on rodents which showed that there is a strong relationship between the hearing and startle threshold meaning that compromised auditory function leads to higher startle thresholds and therefore higher received levels are needed to elicit the reflex.

The results from the field trials suggest that flight responses occur over distances of at least 60m-100m distance (sound source). Flight distances seem to depend on received levels and would probably be higher in case of higher source levels. Animals might also flee over higher distances in open habitats (rather than my test situation around sand bars). The field trials also give evidence that the area that is entirely avoided by seals coincides with received levels that just exceed the startle threshold.

The data challenge current marine mammal noise exposure criteria for pulsed sound which are based on temporary threshold shift (TTS) data. I suggest that noise exposure criteria for pulses should be based on the startle reflex threshold rather than TTS since as shown by my data animals can sensitise to noise and show extreme avoidance responses even though no TTS is caused. A noise sound pressure level of 164 dB re 1µPa (peak) is suggested for pulsed mid-frequency noise in pinnipeds but startle reflex thresholds should be measured for more species using more sensitive electro-myographic methods. If sensation levels obtained from the seal data were applied to *odontocetes*, a conservative estimate would suggest a startle reflex threshold as low as 125-130 dB re 1µPa in the ultrasonic range. The argument that the concept of the sensation level does not have an experimental justification in animals (Southall et al., 2008) is incorrect in the context of the startle reflex (see Ouagazzal et al., 2006). However, it is possible that special adaptations in the auditory system of echolocating odontocetes have altered the startle reflex. Therefore, further experiments on such species are necessary.

The observed behavioural responses also hold great potential for startle stimuli to be used in acoustic deterrence systems. The low duty cycle as well the possibility to use fear conditioning to minimise the exposure to startling sounds would be desirable from a conservation point of view. In addition, species differences in hearing thresholds could be exploited to create sound stimuli that would exceed the startle threshold in one species but not in another.

From an evolutionary point of view it is suggested that the startle reflex serves in increasing an animals' propensity for flight. Flight responses can be considered a SSDR (see Bolles, 1970) in seals and short sound pulses seem to act as an appropriate unconditioned stimulus to elicit such responses.

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Chapter 6

Target-specificity and efficiency of a new startle-based acoustic deterrence system on a fish farm

Introduction

A variety of different anti-predator methods has been developed for use on fish farms. However, none of these methods is without problems (Hawkins, 1985; Quick et al., 2004). Predator deterrent methods should be evaluated by balancing costs, effectiveness, ease of use and conservation concerns (e.g. unintended impact on marine wildlife). For instance, tensioning nets or adding a predator net may be a cost-effective way of preventing seals from sucking fish through the nets but can be difficult to maintain if tidal currents are strong (Ross et al., 2001). Predator nets also seem to vary in effectiveness (Hawkins, 1985) and can lead to tangling of diving birds and other wildlife if nets are badly maintained (Ross et al., 2001). Population control or targeted lethal shooting are also problematic from a conservation point of view. If predator populations are critically endangered, predator population control can be considered ethically questionable and seems to be even ineffective in some areas (Pemberton & Shaughnessy, 1993). Therefore, acoustic deterrent devices have often been considered a benign way of solving the problem. However, while being somewhat effective against *phocids* (Mate & Harvey, 1987) animals generally seem to habituate to the sound. The time frame within which habituation occurs can be highly variable ranging from weeks to years in different regions (Rivinus, 1987; Mate et al., 1987; Harvey & Mate, 1987). Deterring *otariids* seems to be even more difficult: Acoustic deterrent devices used to protect salmon runs (NMFS, 1995) and fish farms (Norberg, 1998) had little effect on sea lions although in some areas recruitment of new individuals was successfully prevented (NMFS, 1995). In one case even a “dinner bell” effect attracting sea lions to the sound source has been reported (Jefferson & Curry, 1996) implying that the originally aversive stimulus had become a conditioned reinforcer.

Acoustic deterrent devices have been criticised for their potential impact on other marine wildlife, in particular cetaceans with sophisticated high-frequency underwater hearing. While some authors have argued that the the possibility that certain ADDs could even damage the hearing system of cetaceans cannot be ruled out (Taylor et al., 1997; Gordon & Northridge, 2002) experimental evidence for adverse impacts of ADDs on cetaceans only exists on the level of behavioural avoidance responses and habitat exclusion. Olesiuk et *al*. (2002) showed that harbour porpoise (*Phocoena phocoena)* sightings in the Broughton Archipelago (British Columbia) dropped to 10 % of the expected value at ranges up to 2500 and 3500m from an operating ADD. In another study using an Airmar ADD, porpoises were excluded completely from an area extending up to 645 m from the device and numbers were significantly lower in an area of up to 1.5 km. In comparison, animals were seen within 10m of the device in silent control sessions (Johnston, 2002). Morton & Symmonds (2002) reported a dramatic decrease in killer whale (*Orcinus orca*) sightings in the Johnston Strait/Canada after ADDs had been introduced on fish farms and a recovery of sighting rates after fish farmers stopped using ADDs. Their study covered a period of 15 years and therefore indicates that killer whales, in contrast to seals, did not habituate to ADDs. Morton (2000) found that the abundance of Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) decreased after ADDs were introduced in the same area. These studies all show that the use of conventional ADDs against seals is likely to be problematic in areas that are of importance for toothed whale populations.

One possibility to mitigate impacts on high-frequency hearing specialists (e.g. toothed whales) is to decrease the perceived loudness of the sound. In humans the contours of perceived equal loudness (iso-phones) run parallel to the hearing threshold within the most sensitive hearing range but are closer together at the high and low frequency edges of the hearing range (Robinson & Dadson, 1956; Fletcher & Munson, 1933). Equal loudness contours are also flatter at very high sound pressure levels (110 dB). Although strictly speaking the human ear processes sound as a potential function of sound pressure (Stevens, 1956) a rough approximation is to assume that sound pressure levels that exceed the hearing threshold by a similar amount in dB will cause a similar perception of loudness. These sensation levels are expressed as the sound pressure level in dB above the hearing threshold. As pointed out in chapter 2 sensation levels caused by ADDs in odontocetes could be reduced by approximately 40 dB if a frequency band between 500 Hz and 2 kHz was chosen while sensation level in seals would only be slightly lower than those caused by current ADDs.

In this study I investigated the efficiency of such low frequency sounds on seal and cetaceans when played from an active salmon farm off the West coast of Scotland. The site allowed us to observe responses in seals (mostly *Phoca vitulina*), minke whales (*Balaenoptera acurostrata*) and harbour porpoises (*Phocoena phocoena*).

The concept applied in this study was to exploit the acoustic startle response (ASR), a simple reflex arc known to be mediated by a small number of neurons in response to sound stimuli that exceed the hearing threshold by 80-90 dB (see Koch & Schnitzler, 1997 for a review). Experiment 1 and 2 from chapter 5 suggests that the reflex leads to a contraction of skeletal muscles, causes an interrupting of foraging behaviour and initiated flight response in the tested seals. While the magnitude of the startle response (usually measured by muscle activity) is subject to habituation, flight responses in the pool experiment (chapter 5) increased and animal apparently sensitised. Using the ASR is not only advantageous because of the high stereotypy of behavioural response of the target species (seals) but also because all dramatic behavioural avoidance responses should be limited to a very small area around the device where received levels exceed the startle threshold. In addition using very short and infrequent noise pulses will greatly reduce the duty cycle and therefore decrease noise pollution and limit any unintended impact on hearing or behaviour in non-target and target species.

Methods

Study site

The study site was located in the Northern Sound of Mull off the Isle of Mull on the west coast of Scotland/UK. Experiments were conducted on the fish farm in Bloody Bay which is owned by Scottish Sea Farms Ltd (see fig 1). The fish farm was stocked with 5 cages containing young salmon ranging in size from 20 cm-40 cm. The fish farm cages were about between 11 and 15m deep and had a diameter of 25m. There was a small circular walkway around each cage but cages were not connected by walkways. Maintenance work was carried out with RHIBs. A barge was permanently moored at the north-western end of the farm serving as a food storage depot and general base. The acoustic deterrence device was placed on one of the sea-side cages (see figure 1).

Seal predation history

The fish farm reported harbour seal (*Phoca vitulina*) predation during the experimental period as indicated by typical bite wounds on salmon that were found dead at the bottom of the cage. Some fish showed bite wounds that were concentrated on the belly (with internal organs pulled out) and were therefore consistent with seals attempting to bite and suck fish through the net as has been previously reported in the literature (Ross, 1988). Due to the small mesh size of approximately 5cm these were probably relatively unsuccessful but nonetheless lethal attempts. There was one report by a staff member of a seal having possibly entered or trying to enter the cage.

The site manager (Andrew Tomison) reported that seal predation has been a problem in previous years and licensed lethal shooting was used to manage it. Seals have also been observed having entered the cages in previous years. The fish farm has never used an acoustic deterrence device before due to licence restrictions from governmental organisations (Scottish Natural Heritage) and lobbying of local NGOs based on the assumption that the area is a porpoise hotspot (oral information by the site manager)

Playback equipment

The acoustic deterrent device consisted of a Lubell 9162 loudspeaker (Lubell Labs Inc, Columbus, Ohio), a Cadence Z9000 stereo high-power car amplifier (Cadence Sound Systems, Inc), a Panasonic SL-S120 CD player and a car battery installed in a waterproof aluminium box. The aluminium box was strapped to the walkway of the fish farm and the transducer was deployed at 17m depth. This was about 2m below the deepest part of the cage in order to avoid sound shadow effects by the fish in the near field. The source level of the acoustic deterrent device was adjusted to 180 dB re 1µPa (rms) @ 1m (for description of level measurements see below). The playback sounds were synthesised using Cool Edit pro 1.2 (Syntrillum Software Corporation). The sound stimulus was a 200 ms long, approximately 2-3-octave-band noise pulse with a peak frequency of 950 Hz (-20 dB points at 500 Hz and 1.9 kHz). The rise time of the signal was 5 ms. The -10 dB-bandwidth was 800 Hz and ranged from 650 Hz to 1450 Hz. The signal was created by shaping white noise synthesised in Cool Edit Pro with an 8th order Butterworth band-pass filter and an envelope function. Since white noise is created randomly I synthesised 5 different pulses that were arranged pseudo-randomly in the playback track. This was done to ensure that a behavioural response is due the general properties of the signal and not the noise characteristics of one particular pulse.

As pointed out in chapter 5 there may be a link between startle amplitude and the strength of avoidance responses. Therefore, the protocol was designed to decrease the likelihood of strongly diminishing startle amplitudes. Habituation of startle amplitude depends on many factors including stimulus intensity but also stimulus presentation schedule (Davis, 1984). Habituation training with presentation schedules using variable inter-stimulus intervals leads to higher startle amplitude in post-training tests compared to training on a regular stimulus interval schedule (Davis, 1970). Therefore irregular inter-stimulus intervals were chosen. The noise pulses were played at intervals ranging from 2s to 40s with an average of 2.4 pulses per minute. The effective duty cycle of the acoustic deterrence device was 0.08 %. In order to make the sound pattern less predictable the signals were arranged digitally in Cool Edit into 4 different playback tracks each of which was 1.5 h long. These playback tracks were assigned to different playback days, burnt on CD and played in loop mode from the CD player.

Experimental design

Experiments were carried out in sea states of less than 3 in June/July 2007. The aim was to have sound exposure and control observation period blocks of about 4 hour length on separate days. A day was assigned to be a playback or control day in a pseudorandomised order. However due to engine problems with the boat that was used deploy the device there was a long series of control days in the beginning. The order of sound exposure (SE) and control (CO) days was as follows: 8 CO, 2 SE, 1CO, 2 SE, 1CO, 3 SE, 1 CO, 3 SE, 1 CO, 1 SE, 1 CO, 3 SE, 3 CO, 2 SE. The full length of 4h observation blocks was only achieved on 52 % of the observation days due to adverse weather conditions on the rest of the days. If sea state had built up to Beaufort 3 or if a strong wind/gale warning required us to leave the observation post the observation period was cut short. It was attempted to add the missing time to the observation period on one of the following days. Average observation periods were 3.5 h (SD=0.96) on control days and 3.4 h (SD=0.94) on days with sound exposure. The longest observation period was 5 hours, the shortest 1.5 hours. This protocol resulted in a total of 113 hours of observation with 58h during sound exposure and 55h during control periods.

Sea state was not significantly higher (t-test, t_{32}=1.611, p=0.117) on sound exposure days (mean=2.12, SD=0.65) compared to control days (mean=1.71, SD=0.65). The average start time of the observation period on each day did not differ (t-test, t_{32}=1.204, p=0.238) between control (mean 10:34 BST, SD=2h 17 min) and playback sessions (mean=11:01 BST, SD=2h 15min). Since the tidal cycle shifts from day to day but start times for both treatments did not differ significantly this would also indicate that the average position within the tidal cycle was not fundamentally different between the two treatments. Finally, average tide height did also not differ significantly between control and sound exposure days (t-test, t_{32}=0.688, p=0.496).

Theodolite tracking and visual scanning

Visual scans were conducted by two observers either by naked eye (focusing on the area around the fish farm) or with 10X50 binoculars. One observer was scanning by eye while the other observer was using binoculars. If one of the observers detected a group he tried to locate the group with the theodolite and, if successful, started logging surface positions to track the group. A group was tracked until no resurfacing occurred 15 min after the last surfacing had been logged. Group and track ID was therefore defined as a consecutive line of surfacings that were not separated by more than 15min. This was done to avoid over-inflating group numbers while taking into account that on many occasions a group could have easily left our observation area within a 15 min time period. The other observer continued to scan the area by naked eye or with binoculars and if he spotted another group or species he would indicate the new position to the tracker on the theodolite who would then try to log surfacings of both groups alternatingly. If a group was lost (no resurfacing with a 15min period) the tracker returned to scanning. The observation area included the whole Northern Sound of Mull that was visible from our observation post (see maps in results).

Measurements were taken with a Topcon DT-102 digital theodolite. The horizontal angle was set to zero using a reference point at Rubha nan Ghal lighthouse (two thin metal bars forming a small cross directly in front of the light bulb). The position of the theodolite station was measured with a handheld GPS receiver on 2 days with low cloud coverage. The bearing from the theodolite station to the lighthouse was calculated using the known position of the theodolite and the known position of the lighthouse. The altitude of the theodolite station was calculated using two trig points (Ben Hiant & Cnog na Staing) and the known height of the light of Rubha nan Ghal lighthouse. If possible the vertical and horizontal angle to the two trig points and the lighthouse were measured daily although on some occasion low level cloud cover obstructed the trig point on the summit of Ben Hiant. The results from the daily measurements were averaged for each reference point and then a mean was value was calculated. This resulted in a measured altitude value of 73.5m above OS survey datum for the theodolite station. In order to confirm that this value was in the right order of magnitude I also measured the distances (with a Bushnell Yardage pro 1000 laser-rangefinder) and vertical angle (theodolite) to 3 mooring buoys of the fish farm and calculated the height of the observation station using simple trigonometric equations. This less accurate measurement resulted in a value of 72.5 m (corrected for tide height).

All data were processed in Pythagoras 1.2.15 (Glenn Gailey & Joel Ortega, Galveston). A tide height table for Tobermory Bay (10 min intervals) was exported from POLTIPS 3.2 (Applications Group, Proudman Oceanographic Laboratory) and uploaded in Pythagoras. The surface positions were then calculated in Pythagoras taking tide height into account. These positions were then exported and distances between the surface positions and the transducer were calculated using the Vincenty formula (Vincenty, 1975). The position of the transducer was measured every day and several reference points on the fish farm were mapped for plotting purposes (see. fig 1).

Sound field measurements

The sound field was measured using a calibrated Bruehl & Kjaer 8103 hydrophone connected to a Bruel & Kjaer charge amplifier 2635 operating in acceleration mode. This was done outside of experimental exposure or control periods. The output from the charge amplifier was recorded through the line-in of a Toshiba Satellite Pro laptop with the in built sound card (SoundMax Digital Audio). The sound card was calibrated using a Thurlby Thandar TG 230 signal generator. The output from the signal generator was confirmed with a Tektronix TDS 3022 digital oscilloscope capable of doing accurate peak-to peak and rms voltage measurements. The measured frequency response of the sound card was flat (+/- 1.5 dB) in the frequency band from 70 Hz to 15 kHz. The amplification of the sound card was calculated by dividing the actual recorded voltage by the known voltage of a calibration signal generated by the charge amplifier. The voltage of the calibration signal from the charge amplifier was also verified by measurements with the digital oscilloscope. Recordings were made using Cool Edit Pro 1.2 software (Syntrillum Software Corporation). Root-mean square (rms) and peak to peak (p-p) voltages of the recorded sound and calibration signals were measured in Avisoft SAS Lab Pro v 4.32 (Avisoft Bioacoustics, Raimund Specht, Berlin). The sound pressure (SP) was calculated from the corrected recorded voltage output from the charge amplifier (e.g. when the gain of the charge amplifier is set to 1mV the amplifier will output 1mV per Pa; knowing the gain of the sound card this can be calculated back to sound pressure measurements). Sound pressure levels (SPL) were calculated as SPL=20log (sound pressure/1µPa).

The source level of the acoustic deterrence device was measured at sea in 20m deep water at 2m depth from a drifting Zodiac. Transducer and hydrophone were deployed

1.7 m apart. The source level at 1m distance was defined as the measured received level plus an assumed transmission loss of 4.61 dB (20* Log (1.7m)). The average of 20 measured pulses was taken to calculate the source level. The free field measurements of the sound field around the fish farm were done with the hydrophone deployed at 2m depth from a drifting Zodiac with the engine switched off. The position of the hydrophone was measured with the theodolite for distances of up to 1000m. For distances of more than one 1 km a handheld GPS receiver was used. This was done because the theodolite measurements are likely to be more accurate at short distance. The locations of the measurements (particularly at distances >500m) were chosen based on where animals had been regularly sighted during both control and sound exposure observations.

Data analysis and sample size

Observations were carried out on 34 days but two days were excluded from analysis due to sea states higher than 3 resulting in a total number of 32 observation days (16 days sound exposure; 16 days control). 58 hours of observations were carried out on control days and 55 hours on sound exposure days resulting in a total number of 113 observation hours. Harbour porpoises were observed on 19 out of the 32 days. Overall 59 porpoise groups (136 animals) were tracked. Seals were observed on 22 out of the 32 observation days with a total of 53 animals tracked. Seals could not always be identified to species level, however, all identified individuals were harbour seals.

A variety of response variables were used for analysis. I calculated the closest observed approaches defined as the surfacing closest to the transducer and the average distance from the transducer for all surfacings within a trackline. Porpoise sighting data were analysed with both groups and individuals as the unit of analysis. This was not done for seals since there was only two occasions when two seals were seen interacting and both animals clearly behaved not independent of each other (this was only one event which was logged as one encounter and not two seals). Minke whales (*Balaenoptera acutorostrata*) were always seen alone. Data was analysed in distance bins of 0-250m, 250m-1500m and more than 1500m. The distance of 1500m was chosen to enable us to compare our data with the study by Johnston (2002) while the 250m were chosen to represent an area where it would be desirable to exclude seals from with respect to depredation. As a measure of sighting density I calculated the percentage of hours with animals present based on the closest observed approach per track.

In order to answer certain questions related the observed behavioural responses some of the response variables were analysed for different distances from the sound source (e.g. 0-500, 0-1000m etc). Since this constituted a case of multiple testing on the same data set a Boneferroni adjustment was applied. However, it should be noted that Boneferroni adjustments of the significance level have been criticised strongly and some authors argue that even statisticians disagree when they should or shouldn't be used (Nakagawa, 2004). I did therefore restrict adjustments to the mentioned classical case but did not use them if tests were based on different response variables and only one test was carried for a given response variable.

Results

Basic sighting data

The basic sighting data for control and sound exposure periods is summarized in table 1. The detection function for all sightings and control sessions is given in appendix 3. The detection function for porpoise sightings showed a bimodal distribution with one peak at distances of less than 500 m and a second peak at distances between 1500m and 2500m. The bimodal distribution of harbour porpoise sightings is mainly due to the fact that there were more tracks with more surfacings closer than 500m during sound exposure. Porpoise group size did not differ significantly (t-test, t =0.46, p=0.50) between sound exposure (mean 2.41+/-0.19) and control periods (mean 2.12+/- 0.20). Group sizes ranged from 1-5 animals in both treatments.

	Harbour porpoises		Seals		Minke whales	
	C	S	C	S	C	S
Groups/tracks	32	27	35	18	1	6
Individuals	71	65	37	18	1	6
Surfacings logged	122	167	86	26	4	25

Table 1: Basic sighting data for all three observed species for control observation periods (C) and sound exposure (S)

The detection function for seals did reflect the expected shape with a rather sharp drop off for distances more than 1000m. Seals were always sighted as single individuals except for two occasions during a control session when two seals were seen interacting. Minke whales have never been detected closer than 1km from the farm during any of the treatments and were always single animals.

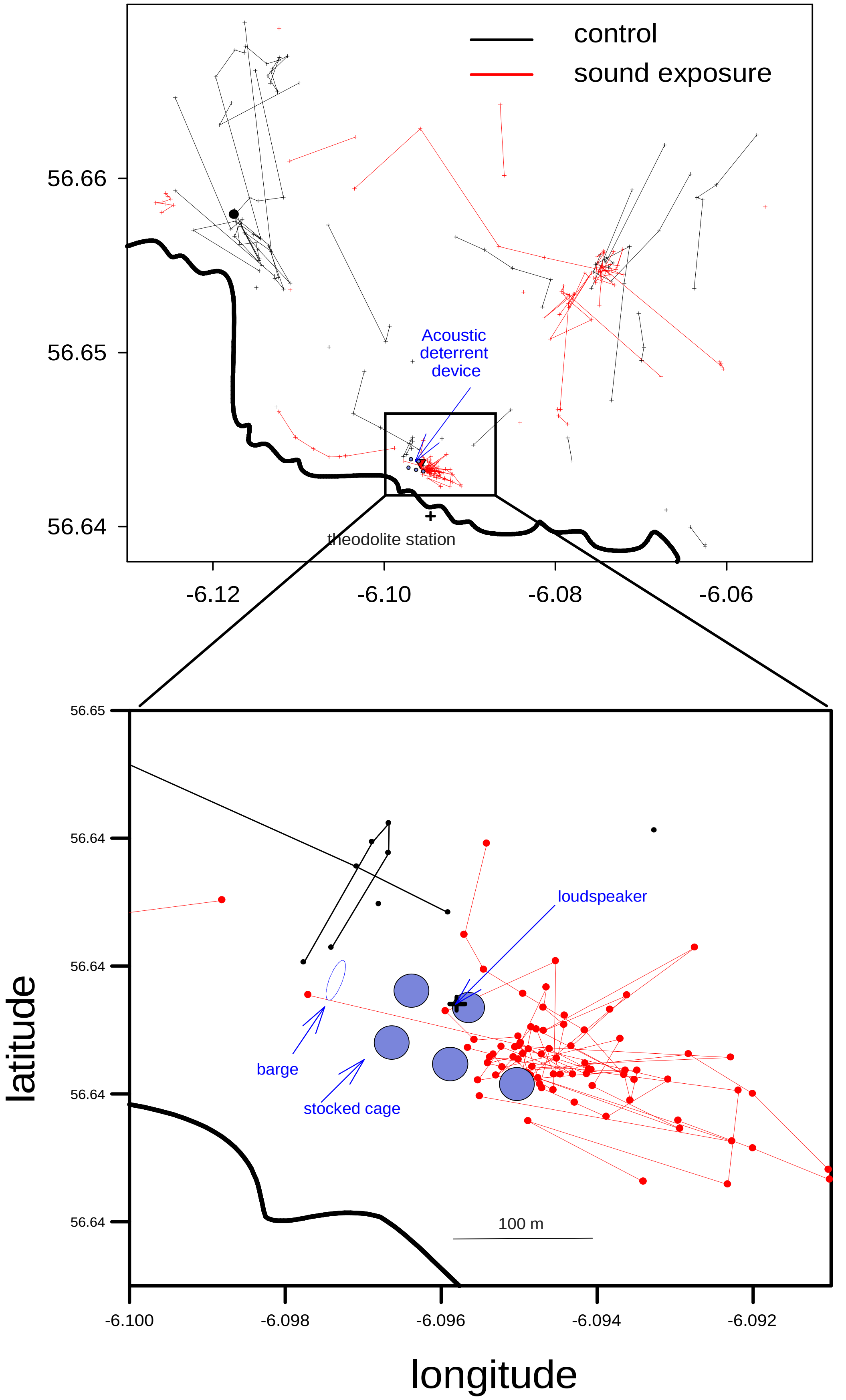

Figure 1: Map showing all porpoise track-lines for sound exposure (red) and control observation periods (black)

Harbour porpoises *(Phocoena phoecona)*

Tracking maps

The map of all harbour porpoise tracks shows a similar pattern of distribution for control and sound exposure periods at distances of more than 1000m (fig 1). There were two hotspots of sightings in a similar area during sound exposure (red tracks) and during control sessions (black tracks); one in the central Northern Sound of Mull approximately 1.5km northeast of the fish farm while the second one is located north of Ardmore Point (56° 39' .378' North, 006° 07 .703' West) north-west of the farm. The concentration of sightings north-west of the fish farm was more prominent during control sessions but more porpoise tracks were logged north east of the farm during sound exposure. The close-range map (figure 1, lower panel) shows a striking pattern with substantially more and longer tracks close to the farm when the device was switched on. Five different porpoise tracks with 43 surfacings closer than 100m of the device were observed during sound exposure. In contrast only two tracks with substantially 3 surfacings at distance closer than 100m were logged on control days. All sightings closer than 500m were groups of two animals. In most cases the group could be identified as consisting of two animals with a substantial size difference making it likely that these were mother-calf pairs. These mother-calf pairs (or possibly single pair) were seen swimming between the fish farm cages when the acoustic deterrent device was switched on.

Relative abundance by distance bin

The relative distribution at different distances from the sound source was measured by comparing closest observed approaches and average distance per track for porpoise groups and all observed individuals (fig 2). Marginally more porpoise (group) sightings were observed at distances of less than 250m during sound exposure. In contrast there were slightly less porpoise tracks at distances of more than 1.5km during the sound treatment. The Freeman-Halton-Fisher exact was used to test if porpoise/group count ratios (sound exposure versus control sessions) changed as a function of distance. This was based on the assumption that if sound exposure had an effect on porpoise distribution this would be expected to show up as a reduction of porpoise sightings at close ranges during sound exposure while the shift should be less pronounced or non-existent at higher distances where received levels are lower.

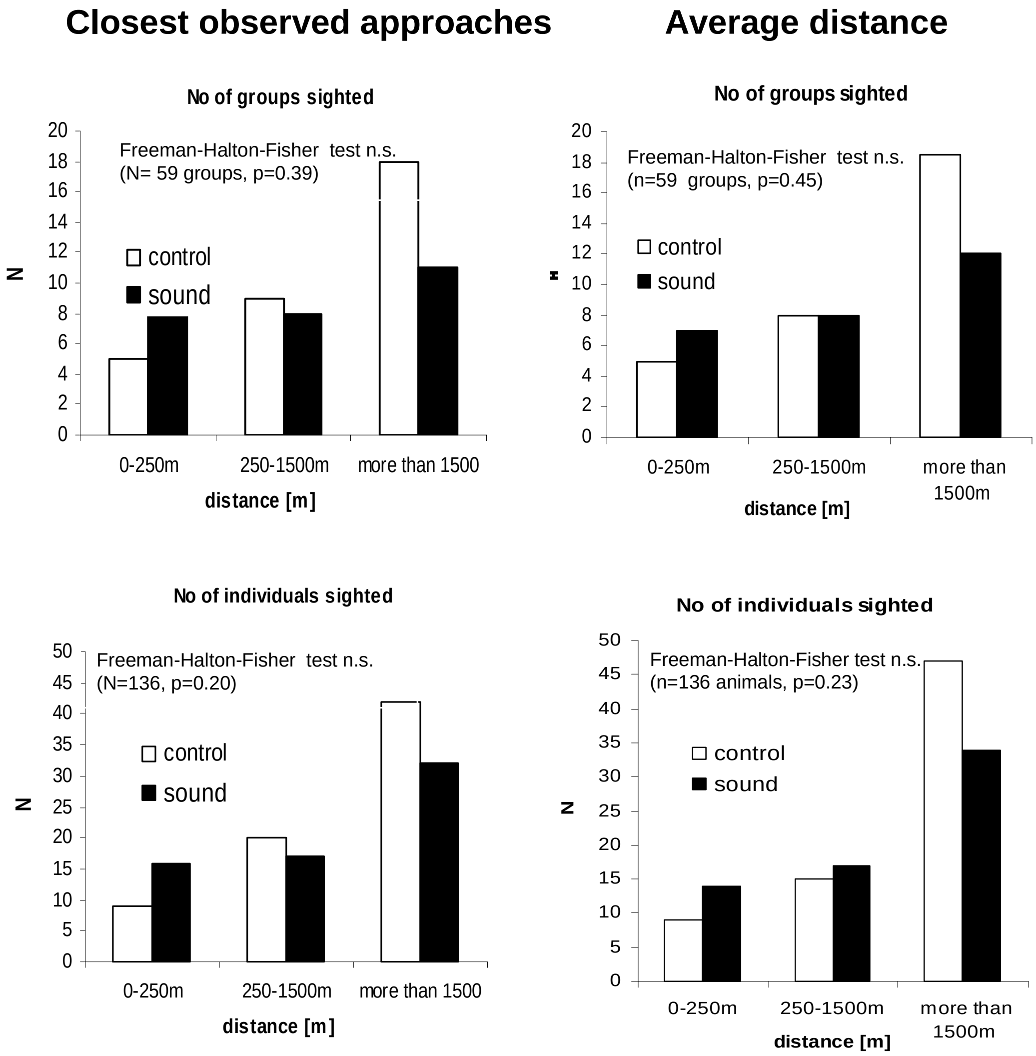

Figure 2: Overall number of sighted porpoises (lower row) and number of sighted groups (upper row) per distance bin. The left column shows the sightings per trackline calculated from the closest observed approach distance while the graphs in the right column are based on average distances per trackline.

There was no statistically significant ($p<0.05$) shift in relative abundance as a result of sound exposure for any of the tested response variables shown in figure 2.

Closest observed approaches

The closest ever observed approach (COA) to the transducer was a mother calf pair surfacing at 8m distance while the ADD was operating at full source level. The closest observed approach during a control session was 32m. Median closest observed approaches did not differ significantly between sound exposure (median=85m) and control sessions (median=220m) for distances up to 1000m (Mann-Whitney U test, n=19, U=60, p=0.221). There was also no significant difference between control (median=730) and sound exposure (median=458) when distances up to 1.5km were included in the analysis (Mann-Whitney U test, n=30, U=123, p=0.647).

Percentage of hours with porpoise sightings

The percentage of hours during which at least one porpoise group was present within 1.5 km from the device did not differ significantly between the sound and control treatments (see fig 3, Fisher's exact test, n=113, $p<0.05$). This was the case for distances up to 500m (p=0.785), up to 1500m (p=1.0) and for the whole area scanned (p=0.561).

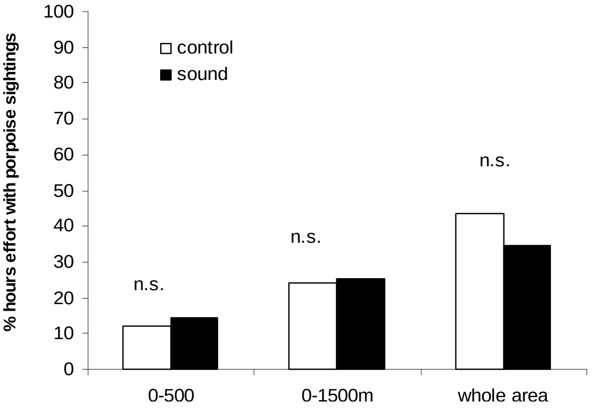

Figure 3: Percentage of hours with porpoise sightings for different observation areas

Track lengths

Similarly, porpoise track lengths measured as the number of surfacings within 1500m from the sound source did not differ between sound exposure (median=2) and the control (median=1.5) treatment (Mann-Whitney U, N=30, U=84.5, p=0.226). The longest track (22 surfacings, 1h 34 min) occurred during sound exposure in the vicinity of the fish farm.

Mean no of porpoise per day

The mean no of porpoise groups sighted per day in an area closer than 1500m was calculated (fig 4). There was no significant difference between the control and sound exposure treatment (t-test, t_{32}=0.799, p=0.43).

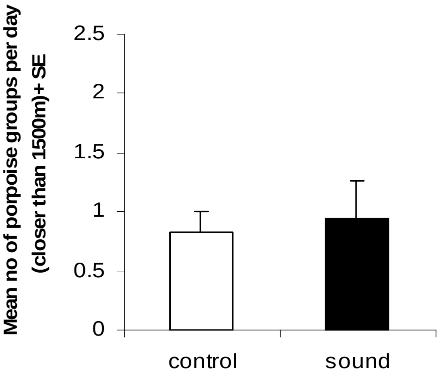

Figure 4: Density of porpoise sightings within an observation area extending up to 1500m from the sound source.

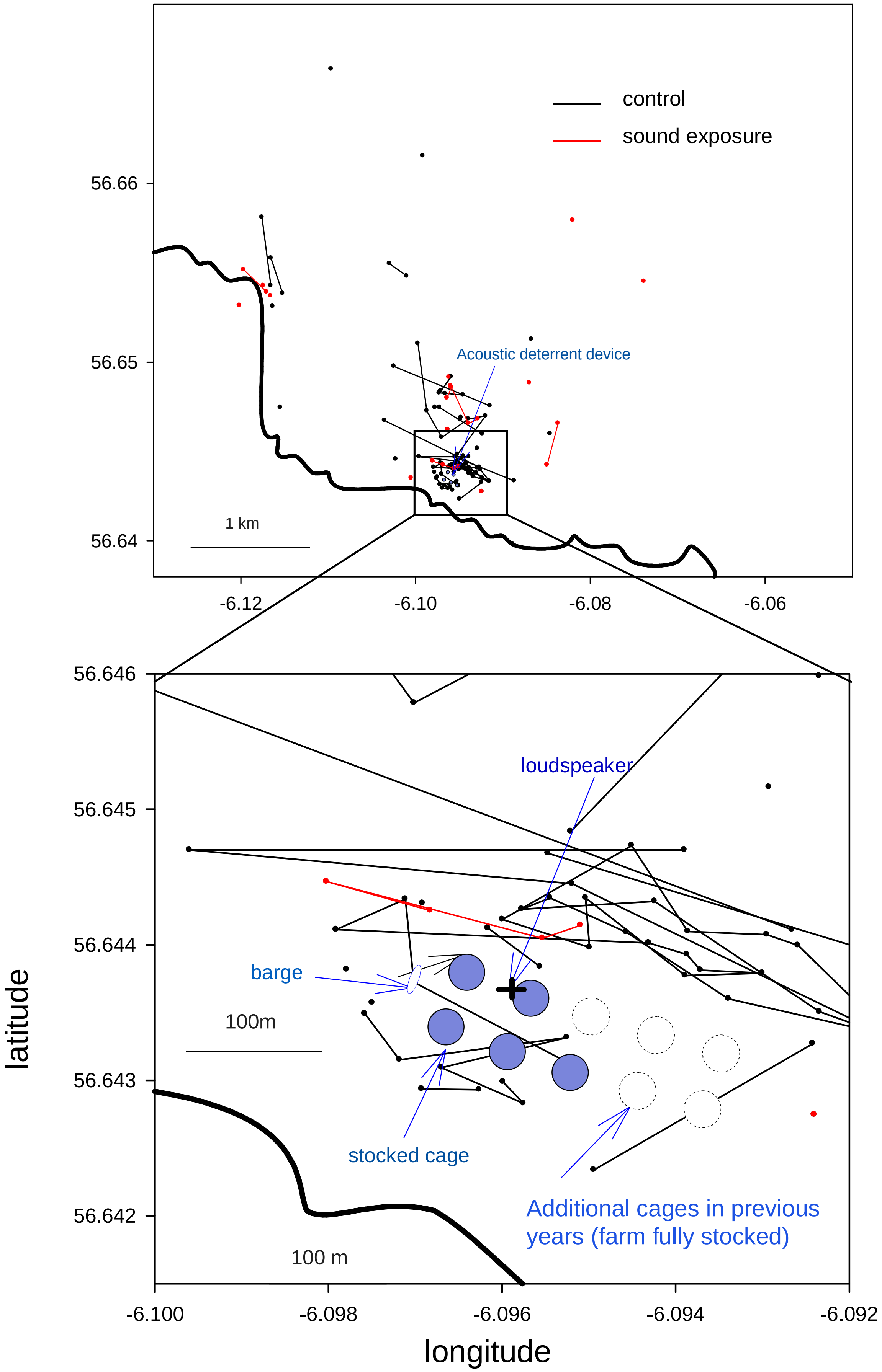

Figure 5: Seal tracks for sound exposure (red) and control observation periods (black)

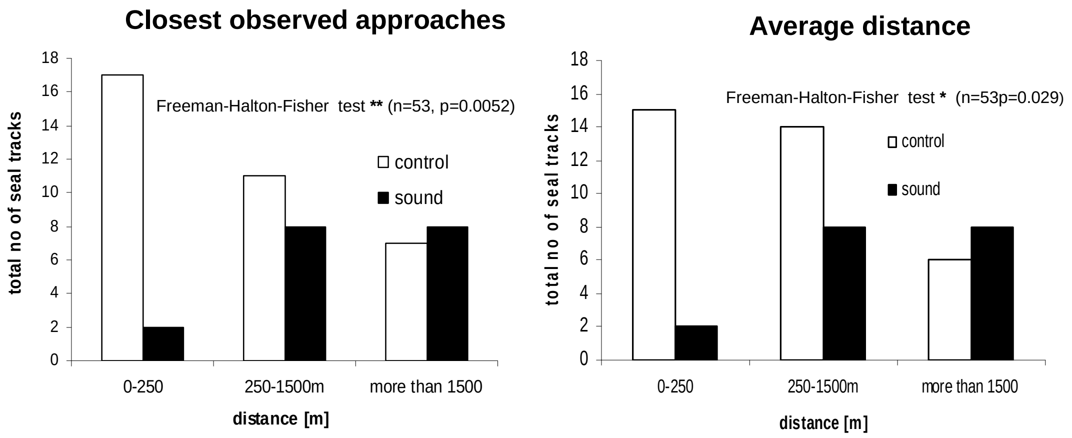

Figure 6: Number of seal tracks per distance bin based on closest observed approaches and mean distance per track

Seals

Tracking maps

The tracking maps (fig 5) for seals show less seal tracks during sound exposure (red lines) compared to control session (black lines). This becomes particularly obvious for distances of less than 250m. In the closest distance bin 17 different tracks were logged during the control treatment while only two tracks occurred during exposure. The map also shows cages from previous years since seals that may have had experience in foraging on the farm might have known these locations.

Relative abundance by distance bins

In contrast to the pattern seen for porpoises there was a dramatic decrease in seal numbers at distances less than 250m during sound exposure (fig 6). The significant Freeman-Halton-Fisher exact test ($p<0.05$) shows that the ratio of seal sightings between sound exposure and control session was different in the three distance bins.

Closest observed approache distances

The median closest observed approache distances calculated over all tracks differed significantly (Mann-Whitney U test, n=35, U=70, p=0.045) between sound (median=315m) and control sessions (median=120 m) for sightings at distances of less than 1000m. There was no significant difference in the median closest observed approaches between control (median=144m) and sound sessions (median=315m) if all tracks within an area of 1500m from the acoustic deterrent device were included in the analysis (Mann-Whitney U test, n=38, U=102, p=0.208). The closest ever observed approach of a seal was 22m for control sessions and 50m for sound exposure days.

Percentage of hours with seal sightings

The proportion of hours with seal sightings during sound exposure and control periods are shown in figure 7. Seals were present in an area within 500m of the ADD during a third of the observation time (32 % of all hours, 21 hours) on control days. In contrast seals were only sighted during 8 out of 55 observation hours (14.5 %) when the ADD was switched on. This represents a significant decrease of the time seals were seen around the fish farm during sound exposure (Fisher's exact test, n=113, p=0.016). There was also a significant difference in the percentage of observation hours within which seals where sighted at distances up to 1500m distance (Fisher's exact test, n=113, p=0.0021).

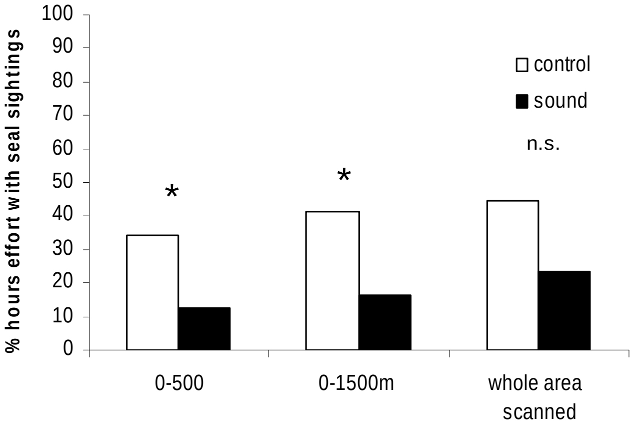

Figure 7: Percentage of observation hours with seal sightings

Differences in seal hours were only marginally significant when compared over the whole area scanned (Fisher's exact test, n=113, p=0.048). However, even when significance levels are Bonferroni-adapted to compensate for multiple testing on the same data set there is significant drop in hours with seal sightings for distance from 0 to 500m and 0 to 1500m during sound exposure periods.

Track length

The number of surfacings per track for distances up to 1.5 km from the transducer did not differ significantly (Mann-Whitney U, n=38, U=161, p=0.451) between sound exposure (median=1) and the control treatment (median=2). However, the maximum track length was much longer during control sessions (27 surfacings) compared to sound exposure (4 surfacings). On one occasion a seal was observed swimming in the vicinity (<100m) of the fish farm for 2 h 5min during the control treatment. The maximum time a seal was observed very close (< 100m) to the fish farm during sound exposure was 21 min.

Seal sighting per day

The median no of seal sightings per day in the vicinity of the fish farm (<250m) was significantly lower during sound exposure compared to control days (see fig 8, Mann-Whitney U test, n=34, U=198.5, p=0.02). The maximum no of logged seal tracks per track per day was 5 on control days and one for sound exposure days.

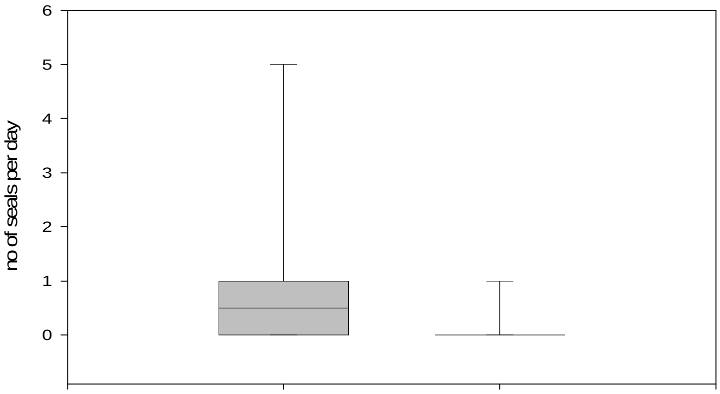

Figure 8: Median no of seals per day closer than 250m from transducer. The box displays the inter-quartile range while whiskers show the 5 & 95% data range.

Minke whales (*Balaenoptera acutorostrata*)

Minke whale tracks are shown in figure 9. The low sighting rate of minke whales with only 7 individuals tracked on sound days and one on a control day does not justify a statistical analysis. The closest ever observed approach for a minke whale was 1109m during sound exposure and 2808m for control periods. The average closest observed approach distance for all tracks was 2391m for the sound treatment while no mean value could be calculated for control days (since only one track was logged on control days). The average track length was 19.3 min (SD =19.4) during sound exposure. The only track that was logged during a control session lasted 10 min. There tracks shown in fig 9 were not directed away from the sound source; the track that contained the closest surfacing was directed into the bay where the fish farm is located.

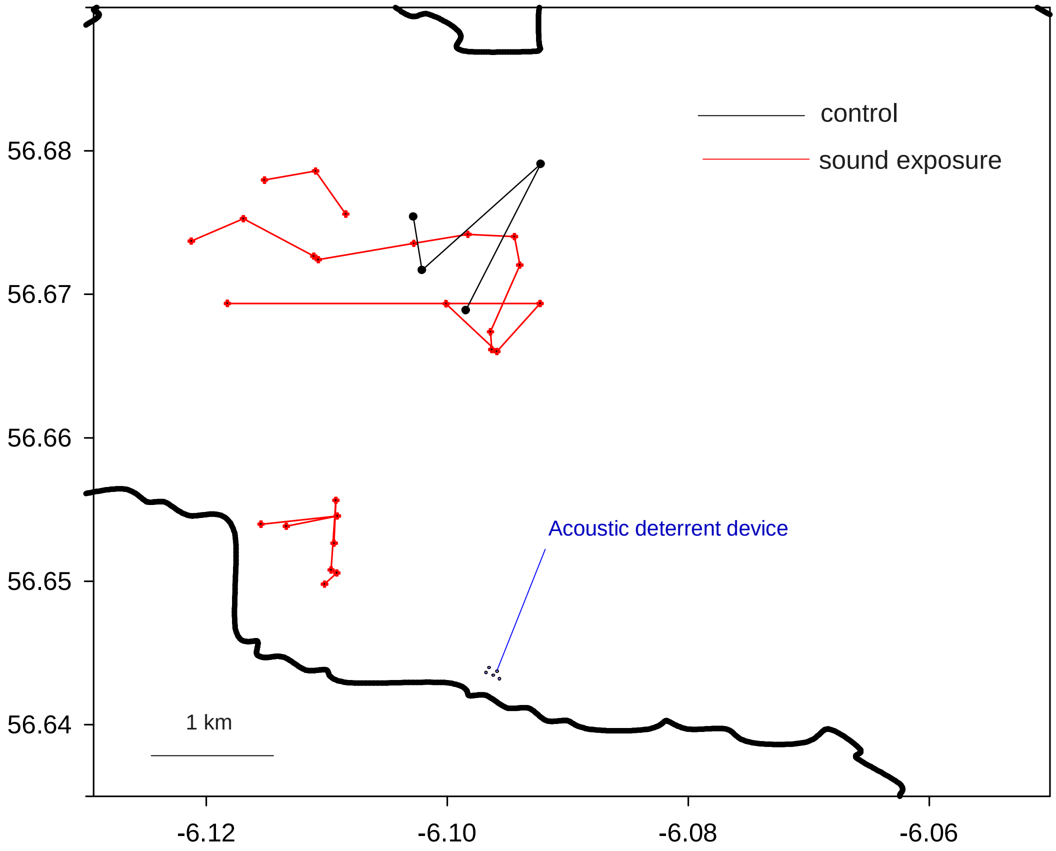

Figure 9: Minke whale tracks during sound exposure and control observation periods

Sound field measurements

Figure 10 shows the measured received levels above the transducer at 2m depth, and 35 data points of measured received levels (2m depth) at different distances. The calculated source level at 17m depth obtained from the measurements with the transducer at 2m depth (180 dB re 1µPa @ 1m) is also shown. The locations of the measurements at higher distances (>500m) were chosen based on where animals had been regularly sighted during both control and sound exposure observations. The sound field measurements indicate that transmission loss was somewhat between spherical and cylindrical spreading. A logarithmic regression line based on the equation

$RL=SL-a*\log_{10}$ (distance from transducer)

was fitted to the measured values for the rms sound pressure level (RL being the received level, SL being the source level at 1m distance and a being a parameter adjusted to the data). The r^2 value of 0.95 indicates a good fit to the data. The parameter a was estimated to be 18.3 indicating that transmission loss was somewhat between spherical (20*log(distance))and cylindrical spreading (10*log(distance)) with a stronger tendency towards spherical spreading. Measurements were conducted at 2m depth but the transducer was projecting at 17m depth. Therefore, true distances to the transducer were calculated for each measured value using Pythagoras' theorem but the actual distance at the surface was plotted in figure 10. Measured received rms-levels at distances of about 250m were around 135 dB re 1 µPa while received levels at 1.5 km distance were approximately 115 dB re 1µPa.

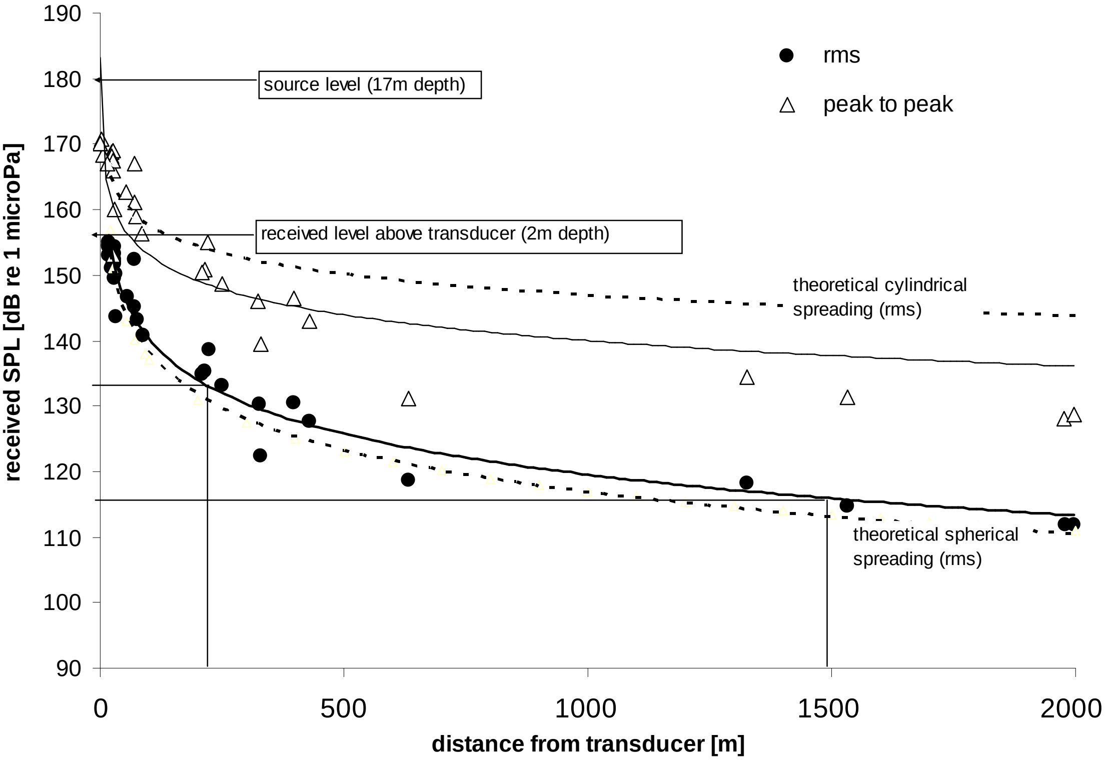

Figure 10: Measured received levels at different distances from the transducer. The equation of the curve fitted to the rms-values is: SPL=SL (180 dB re 1µPa)-18.3 log (distance). Spherical and cylindrical spreading were calculated by SPL=SL-20 log (distance) and SPL=SL-10 log (distance) respectively.

Discussion

Did the new acoustic deterrence device impact harbour porpoise?

Since there was no statistical difference between the control and sound treatment for any of the response variables in any part of the observation areas there is no evidence that the new ADD had any detrimental effect on porpoise abundance and behaviour. The sound field measurements confirmed that measured received levels were a function of distance (best approximated by 18.3 log of distance) and highest received levels clearly occurred in the closest distance bin (0-250m). One would therefore expect that if any behavioural avoidance effect was present it should be strongest in this distance bin and less pronounced at further distances. However, there were, in fact, more and longer porpoise tracks were logged in the closest distance bin during sound exposure compared to control sessions (<250m). Two porpoises were seen surfacing repeatedly within 250m of the operating ADD for periods of up to 1.5h on 3 different days (2 were consecutive days, the last close encounter was 17 days later) . The number of porpoise groups and the overall number of individuals were also similar for the control and sound treatment at distances between 250m and 1500m. In addition, the response variables “number of hours with porpoise sightings”, “number of porpoise sightings per day” and median closest observed approaches were almost identical for the control and playback treatment at distances up to 1500m. The fact that more porpoises were sighted close to the farm during sound exposure could have to do with differences in food availability. There was signs of big schools of mackerel being present on some days (as visible under the surface) in the vicinity of the fish farm; an observation that was also confirmed by divers inspecting the nets. While it seems unlikely that the device had an attraction effect on porpoise the observation might at least indicate that foraging behaviour is not interrupted at distances of more than 10-20m from the device.

Johnston (2002) reported a significant decrease in porpoise sightings per scan and an increase in mean closest observed approaches around a conventional ADD (Airmar Inc.) in an observation area extending up to 1500m from the device. Johnston (2002) reported a modelled received level of 128 dB re 1µPa at the closest ever observed approach distance of 645m during sound exposure. The received level of my device at the same distance modelled by using the equation fitted to the measured values is 128 dB re 1 µPa. The received levels of both ADDs were therefore identical at this distance showing that porpoises respond differently to sounds of the same sound pressure level depending on the frequency band used and differences in the duty cycle. It is obviously possible that other factors (e.g. differences between populations) played a role, too.

The closest ever observed approach of a porpoise group (a mother-calf pair) in this study was 8m. A received level of 154 dB re 1µPa dB was measured at the spot of the surfacing. This is an actual measured value on the surface which is slightly lower than the predicted values based on the measured source level since the distance from the transducer at 17m depth to the spot of the surfacing is obviously higher than the direct distance at the surface. The harbour porpoise hearing thresholds at the peak frequency of the ADD (950 HZ) is around 82 dB re µ1Pa while the threshold at the peak frequencies of the Airmar ADD (10 KHz) is 53 dB re 1µPa (averaged values from Kastelein et al. (2002) and Andersen (1970)). Sensation levels of signal at the distance of the closest observed approach (8m) in my study would therefore be 72 dB (154dB-82dB). Sensation level at the distance of the closest observed approach (645m) caused by the Airmar ADD in the study by Johnston (2002) would have been approximately 75 dB (128 dB re 1µPa minus 53 dB re 1µPa). Although basing calculations on a single closest observed approach during sound exposure may be problematic it seems that in both studies similar sensation levels apparently had a similar effect on porpoise behaviour. The source level of my prototype device was 180 dB re 1µPa and therefore identical to the Airmar device used by Johnston (2002) l. This may point towards sensation levels rather than absolute sound pressure levels being good predictors of behavioural exclusion zones of marine mammals at sea. This finding is consistent with earlier results from studies on captive harbour porpoise, harbour seals and a striped dolphin (*Stenella coeruleoalba*) that suggested that inter-species variation in behavioural responses to same type anthropogenic noise might be primarily due to differences in their hearing abilities (Kastelein et al., 2006a; Kastelein et al., 2006; Kastelein et al., 2005). Interestingly, the estimated sensation levels at the edge of the exclusion zones are in line with data from humans. Discomfort thresholds in humans, measured by electro-physiological parameters that are indicative of stress were found to be around 70 dB sensation level (Spreng, 1975). The data would be consistent with the assumption that loudness perception in *odontocetes* might roughly follow similar principles as in terrestrial mammals and humans.

There were slightly more sightings during control days compared to sound exposure days at distance of more than 1500m. Given that visual detection of porpoise at these distances is extremely difficult the most likely explanation could be the fact that sea state was slightly higher on sound exposure days (mean=2.1) compared to control days (mean=1.7). Although the average sea state was not significantly different (see methods) there were 7 sound exposure days with sea state of 2 or more while there were only 3 control observation days with similar sea state. While sighting probabilities at lower distances may still be relatively high in condition of sea state 2-3 the occurrence of white caps and shadows on the crests of the waves is likely to lead to a decrease of the sighting probability at high distances (e.g. > 1500m). An alternative explanation could be that there was an observer bias e.g. in the sense that both observers paid more attention to the area around the fish farm on sound exposure days or that observers paid less attention to the remote area on days with higher sea state (maybe unconsciously assuming that it would be quite unlikely to see animals that far away in such conditions). Unfortunately, I was unable to conduct completely blind observations. However, both explanations are less likely since the protocol was standardized and each observer would only start a new scan at close ranges after having finished a scan of the whole area. Finally differences in high distance bins could be a result of sound exposure. Olesiuk et al. (2002) found a significant decrease in porpoise density in response to an Airmar ADD even at distances of several kilometres compared to the expected distribution during a no sound control period. They concluded that the deterrence effect extended over the whole observation area. While the area of the strongest decrease in porpoise numbers (up to 400m) is in fact similar to the study by Johnston (2002) the fact that Olesiuk et al. (2002) found a deterrence effect extending up to several kilometers is striking. The larger deterrence ranges may have to do with differences in shallow-water sound propagation. However, since no modelled or measured received levels were provided it is hard to tell which factors were responsible. Since none of the differences in porpoise number at distances of more than 1500m were significant and received levels around our device dropped off logarithmically it is unlikely that the slightly lower number of porpoises at high distances were due to an effect of sound exposure.

Since distribution and behaviour of porpoises did not change in response to the sound exposure in this study I conclude that it is possible to mitigate odontocete habitat exclusion by ADDs. There are, however, several problems inherent to any theodolite tracking study limiting the conclusions that can be drawn. For instance, it cannot be said for sure how many different groups or individuals were sighted over the whole observation period. In theory it may be possible that all animals (always groups of 2) sighted close to the fish farm were the same two individuals and for some reason behaved relatively insensitive to the sound. However, even if this was the case for close-ranges it is unlikely that porpoise sightings in all areas were caused by so few individuals. The occurrence of several concurrent sightings of porpoise groups in different areas points towards a much larger number of animals present in the study area. Further studies will be needed in other areas to see if the results hold true.

Effectiveness as a seal deterrent

In contrast to the results for porpoises, seal numbers were dramatically reduced during sound exposure. Given that the strong drop of seal numbers occurred at distances of less than 250m while numbers were more or less stable at higher distances one can conclude that the deterrence effect was limited to the vicinity of the fish farm. Although significant differences between sound and control treatment were found for some response variables (e.g. no of hours with seal sightings, fig 7) at distances up to 1500m, the analysis by distance bin as well as the maps clearly show that this was mostly due to a drop in seal numbers close to the farm (fig 6). The closest ever observed approach of a seal was 50m during sound exposure. A received level of 150 dB re 1µPa was measured at this spot. Received levels at 250m distance were in the order of 135 dB re 1µPa (see fig 10). Using data from behaviourally measured audiograms for the harbour seal at 1 kHz the corresponding sensation level for both distances can be calculated as 78 dB and 63 dB respectively (the hearing threshold of a harbour seal at 1kHz is assumed to be 72 dB re µPa based on averages from hearing thresholds measured by Kastak & Schusterman 1998; Terhune, 1988). As presented in chapter 5 (startle), the lowest startle threshold for 1 kHz pure tones measured at a 75% response level was 160 dB re 1µPa although a startle response has twice been seen at a level of 155 dB re 1µPa. Corresponding sensation levels based on the composite audiogram are 88 dB and 83 dB respectively. The sensation level at the edge of the total exclusion zone (78 dB) would therefore be 10 dB lower than the startle threshold. Sensation levels at 250m distance (63 dB) were most likely too low to elicit a startle response. This might indicate that the exclusion zone of approximately 50m could be a result of received levels exceeding the startle threshold but animals might have moved further away before sufacing the first time. However, the noise pulse seems to maintain a moderate deterrence effect even at levels below the startle threshold.

Possible confounding factors influencing the acoustic startle response in a naturalistic setting

While almost no information is available on how environmental factors influence behavioural follow-up responses associated with the startle reflex there is a wealth of information concerning modification of the startle amplitude and response latency in controlled laboratory settings. Unfortunately, it is unknown how these relate to flight responses. It is possible that startle amplitudes are somehow linked to the strength of an avoidance response. If this is the case then the effect of a supra-threshold startle stimulus can for example be diminished by a preceding sub-threshold stimulus (up to 500ms before startle pulse); a phenomenon which is known as pre-pulse inhibition (PPI) (e.g. Hoffman & Searle, 1965; Hoffman & Searle, 1968). Pre-pulse inhibition is however unlikely to be a problem in my study since the minimum inter-stimulus interval was at least 2s. In addition to PPI, the interval between supra-threshold startle pulses influences the response to consecutive pulses. The response to a second startle pulse is about 80% of the original response if stimulus interval is at least 16s (Wilson & Groves, 1973). The average stimulus interval of 25s would therefore be expected to be sufficient to prevent a fast reduction in startle amplitudes. As a third factor, even if inter-stimulus intervals are high, startle response amplitudes are subject to habituation (Moyer, 1963). Finally, varying conditions of background noise can influence startle amplitudes. While continuous, moderate background noise leads to an increase in startle amplitude most likely due to the elevated arousal of the animal, intermittent pulsed noise causes a decrease in startle amplitude (Hoffman & Fleshler, 1963b). Ison & Hammond (1971) showed that startle response amplitudes increased with an increase in background noise from 65-70 dB re 20µPa but a further increase from 75-90 dB re 20µPa caused a decrease in response amplitude (probably due to masking effects). Further systematic studies showed a complex relationship between startle amplitude and background noise with signal to noise ratios of 40-50dB causing maximum startle amplitude when high intensity startle signals (120dB re 20µPa) were used (Davis, 1974). Several noise sources were present in my study including engine noise from RHIBs carrying out maintenance tasks, the generator on the support barge and most importantly a cleaner used occasionally by fish farm staff to scratch algae off the nets. The cleaning device produced broadband noise from 1.8 kHz up to 8kHz with received levels exceeding those of the startle pulse in some areas in the vicinity of the cages. The device was used on both sound exposure and control days. Since most energy of the startle pulse was however concentrated at lower frequencies (1kHz) the device did only cause partial masking. However, partial masking of the startle pulse may have decreased its effectiveness as a seal deterrent on some occasions.

Long-term habituation effects in startle have been observed across playback sessions (with 50 presentations per session) in spite of substantial recovery times (days to a week) separating each session (Davis, 1972). However, responses recovered within playback sessions with a sensitisation effect elevating startle amplitudes towards the end of each session. Pilz & Schnitzler (1996) demonstrated that long-term habituation is not due to a rise of the startle elicitation threshold but a change in the input-output function. They also observed habituation of startle amplitudes within as well as across playback sessions. Given these complex relationships it is hard to predict long-term effectiveness of a startle based acoustic deterrence device, an area which warrants further study. However, if the likelihood of occurrence of flight is linked to sensitisation of response latency rather than startle amplitude then responses should persist long-term. In conclusion, given the results from chapter 5 it seems possible to maintain sensitization in flight responses. This might be the case in particular if stimulus presentation is rare and ideally triggered by an approaching animal.

Problems of using lower-frequency sounds: Potential impacts on low-frequency hearing specialists and sound propagation

This study did not aim to address the question if the use of lower-frequency ADDs can be considered a universal way of mitigating unintended impact of ADDs on non-target species. The study rather tried to provide information on whether habitat exclusion of *odontocetes* can be reduced or entirely avoided by using different signals that have been shown to cause a strong deterrence effect in seals (see chapter 2 and 3). This obviously poses the potential risk to impact other species that are not adversely affected by current ADDs. There are two main concerns when using lower-frequency sounds as a deterrent: 1.) These sounds could cause high sensation levels and therefore strong avoidance responses in low-frequency hearing specialists (e.g. baleen whales or fish) 2.) low-frequency sound experience lower absorption and therefore propagate further and could impact animals at higher distances. In terms of the first concern my data provides some limited information on at least one species, the minke whale. While I cannot exclude the possibility that there is a deterrence zone for minke whales, the data from this study provide no evidence for any impact on minke whales at a distance of more than 1000m. In fact, more and longer minke whale tracks were observed on sound exposure days compared to control days and track directions did not suggest that animals were leaving the area. The received levels at the distance of the closest ever observed approach for minke whale (1109m) was 125 dB re 1µPa. No conclusions with respect to corresponding sensation levels can be drawn, since, in spite of some promising modelling attempts (Parks et al., 2007) no absolute hearing thresholds have been measured for any baleen whales species. In conclusion impact on baleen whales at high distances (>1km) might not be dramatic but avoidance thresholds in response to the startle pulses need to be investigated experimentally.

Many fish species are not very sensitive to sound pressure at frequencies higher than 800 Hz, however, some hearing specialists e.g. herring (Enger, 1967) have high sensitivity to sounds of up to several kHz and would therefore be sensitive to the proposed frequency band. To date, there is no study on hearing damage in fish that used brief, intermittent sound pulses that closely resemble those used in my study. I therefore will try to extrapolate from information in the literature. Sound can impact fish on the level of physical trauma caused by the pressure wave, by causing temporary or permanent hearing damage or on the level of behavioural responses (e.g. avoidance, c-starts or masking). Hastings & Popper (2005) reviewed available literature on the physical impact of pile-driving or explosive blasts on fish. They suggested that a sound exposure level of 188 dB re 1µPa^{2}s and exposure to 1800 pile-strikes is required to “knock” a Gourami (family *Osphronemidae*) unconscious. The sound exposure level of a single sound pulse used in my study of 173 dB re 1µPa^{2}s re 1µPa was substantially lower making it therefore unlikely that any physical impact could be caused even within 1m of the transducer. A direct comparison of pile-driving sounds with the noise pulse presented in my study may be problematic because pile-driving sounds have shorter rise-times and fall in a frequency band where most hearing generalist fish are more sensitive. Since both factors increase the risk of damage caused by a pile driver this would mean that impact of the startle pulse would be most likely less severe.

Smith et al. (2004) found a linear correlation between the logarithm of exposure time and the amount of temporary threshold shift that was caused by experimental exposure to white noise in goldfish. When projecting the line from the correlation down to zero (fig 4 in Smith et al. 2004) one would expect that exposure times on the order of a minute are unlikely to cause a measurable TTS. The sound pressure level used by Smith et al. (2004) was 170 dB re 1µPa. Therefore using 1min exposure to 170 dB re 1µPa as an onset TTS criterion would mean that a sound sound exposure level of 188 dB Pa^2-s re 1µPa might just be acceptable. This would mean that even a hearing specialist sitting right next to my ADD would most likely not experience any TTS as a result of a single exposure to the sounds tested in this study. Impact of longer term exposure is more difficult to predict. Hastings et al. (1996) showed that 1h sound exposure to continuous white noise at 180 dB re 1µPa caused some hair cell damage in oscars (*Astronotus occelatus*) but exposure to the same signal at duty cycle of 20% did not result in any damage. The duty cycle in my study was more than one order of magnitude lower (0.08%). Thus, effects on hearing in fish are not very likely; in particular because all given considerations were based on the worst case scenario which is that specimens of a species with high hearing sensitivity at 1 kHz (e.g. herring) stay as close as 1m of the device for an extended amount of time. As discussed in the chapter 2 it should be that unlike in mammals hair cells in fish can regrow after acoustic trauma (Corwin, 1981, Popper & Hoxter, 1984, Lombarte et al., 1993). It however still possible that even temporary damage could have adverse effects on fitness. In terms of behavioural responses I did not notice any obvious reactions of the farmed salmon (*Salmo salar*). Salmon continued schooling in their usual pattern and did not show any signs of a C-start when close to the active loudspeaker (behaviour was observed on two consecutive days for about 10 min). Anecdotal evidence also comes from divers that were inspecting the cages while the ADD was operating. They reported the presence of schools of Atlantic mackerel (*Scomber scombrus*) that were at distances of 30-200m from the ADD. No audiogram for Atlantic mackerel is available but hearing sensitivity might not be high since this species has no connection between the swimbladder and the otoliths. On the level of behavioural responses a well known reaction to sound is a C-start that involves a brief, directional movement away from the source (e.g. Blaxter et al., 1981). It was suggested that the C-start in herring (*Clupea harengus*) is primarily elicited by sound pressure stimulation of the bulla since disruption of the auditory system increases the response threshold dramatically (Blaxter & Hoss, 1981) and juvenile larvae with a yet non-functioning bullae do not show a C-start in response to pressure stimulation (Blaxter & Batty, 1985). Therefore, the sounds tested here might be able to elicit such a reflex at higher distances from the source where stimulation by particle velocity would be unlikely. Startle responses (C-starts) in herring have been reported to reliably occur at sound pressures of about 15 Pa (Blaxter et al., 1981) which corresponds to a sound pressure level of 143 dB re1µPa. A received level of 143 dB re 1 µPa would only be exceeded at distances of less than 100m. In contrast to the findings for seals (chapter 5), startle responses in fish do not always seem to lead to movement responses away from the sound source. For instance C-start reflexes elicited by airgun signals with received levels of 195 dB re 1 µPa did apparently not cause a larger-scale avoidance response e.g. fish moving away from the reef in cod, pollock and coalfish (Wardle et al., 2001). Obviously a lack of a behavioural response does not mean that exposure to the sound does not have fitness consequences. In conclusion impact on fish seem less likely except for areas where fish species with good hearing have an important habitat within 100m of the farm.This might be the case for some fish farms e.g. where herring spawning ground are located in coastal waters close by. Apart from herring there are other coastal fish species with specialised hearing e.g. American shad *(Alosa sappidissma)* audiograms cover a wide frequency up to 180 kHz (Mann et al. 1997). However, the absolute hearing threshold of shad at 1kHz (120-130 dB re 1µPa) is 50 dB higher than in herring meaning that the species is relatively insensitive at the relevant frequencies. Effects on wild fish need to be considered on a case by case basis and should be experimentally investigated but may not pose a fundamental problem at the suggested source levels and frequencies.

The second concern was the fact that low-frequency sound can propagate over long ranges. Absorption coefficients are in the order of 0.06 dB/km at the peak frequency of the ADD tested in this study compared to 0.7dB/km at 10 kHz, the peak frequency of the Airmar device (absorption coefficients based on Fisher & Simmons, 1977 assuming water temperature of 12 degrees Celcius). The difference at 5 km distance would therefore only be approximately 3 dB.

Conclusions

The main conclusions from this study can be summarized as:

1.) Impact of seal scarers on high-frequency hearing specialists can be mitigated by using lower-frequency, low-duty cycle noise pulses. Given that the duty cycle used in this study is one to three orders of magnitude lower than that of a conventional seal scarer (see chapter 2; table 1) noise pollution would also be dramatically reduced.
2.) High source level (180 dB re 1µPa @ 1 m) but low duty-cycle noise pulses are effective in reducing the number of seals in an area up to 250m around the device and excluding seals entirely from an area up to approximately 50m from it. This was probably due to the fact that received levels were high enough to elicit the startle reflex within this area and most importantly

sensation levels exceeded 80-85 dB. However, the noise pulse seems to have a limited deterrence effect even at lower sensation levels (approx. 70 dB)

3.) Minke whales which are expected to have good low-frequency hearing did not seem to respond strongly to received levels lower than 125 dB re 1µPa. Responses to higher received levels could not be investigated since minke whales never approached the fish farm closer than 1km (even during control sessions)

4.) Responses of fish species (hearing specialist and generalists) should be assessed if these occur in the vicinity of a fish farm that intends to use a low-frequency ADD

5.) The data shows that sensation levels may be worth considering as a predictor for behavioural responses to anthropogenic noise.

6.) Low-duty cycle sounds that elicit startle responses might be an effective deterrent for marine mammals in general while minimising noise pollution and limiting any effect to an area in which received levels exceed the startle threshold of a certain targeted species. Inter-species differences in frequency-dependent hearing sensitivity can therefore be used to specifically target a certain species.

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Chapter 7

General discussion and summary

From psycho-physiology to animal communication: What factors influence an animal's response to anthropogenic and natural sounds?

The described experiments tested the influence of a variety of different factors on marine mammals' responses to artificial and biological sounds. Movements and diving behaviour of *phocid* seals were tested in response to 1.) moderately loud, high duty cycle artificial sounds, 2.) brief, startle eliciting pulses 3.) recordings of natural grey seal calls. In addition responses of harbour porpoises and seals were tested in response to short sound pulses. All three classes of stimuli were tested under comparable conditions. This involved experiments on captive (wild-captured) seals and experiments with wild seals around haulout sites. All captive trials involved some simulation of food motivation. Although this approach might have resulted in a lower likelihood of detecting subtle responses it is advantageous to establish a predictable baseline behaviour against which alterations can be tested. In addition the role of food motivation on behavioural responses could be investigated. The data can therefore be used to elucidate perceptual, physiological, motivational and behavioural factors that are important with respect to an animal's response to sound.

In the first series of experiments three different classes of moderately loud stimuli were tested: Sounds based on a psychophysical model of what makes sound unpleasant in humans (Zwicker & Fastl, 1990), two control sound with assumed neutral properties and sounds of current seal scarers. The tests showed that strong avoidance responses (e.g. prevention of foraging) to all stimuli habituated equally rapidly in a context where the animal is motivated to stay close to profitable food source. However, sound exposure remained efficient in eliciting more subtle changes in dive time and time spent close to the food source over the course of the experiment. It also became evident that different levels of food motivation play a crucial role with respect to fine-scale diving behaviour and movement responses e.g. animals stayed longer close to the loudspeaker and feeding station when no food was provided (but the food source was known from previous sessions). In spite of observed habituation of the strong avoidance behaviour seals minimised time spent close to the feeding station in later playback sessions. The results are therefore consistent with the dual-theory of habituation (Groves & Thompson, 1970) which postulates that a habituation process is also accompanied by a sensitising component. The trials in the field provided data on received levels at which a high duty cycle (50%) sound causes an avoidance response in wild animals. Interestingly, the field experiment revealed that although all sounds were played at the same source level (172 dB re 1µPa @ 1 m) deterrence ranges ranged from 40 to 80m. Sound field measurements confirmed that all sounds transmitted equally well meaning that received levels at which different sounds were effective in causing a deterrence effect differed. Given the different frequency characteristics of the sounds, maximum sensation levels at the edge of the deterrence range were calculated using standard audiograms to measure whether sound parameters other than perceived loudness had an influence. This analysis showed that although differences in sensation levels could in fact partly explain variation in deterrence ranges, sounds that were based on the human model of unpleasantness were more aversive even at lower sensation levels. This gives some evidence for dislike of sounds with high roughness similar to humans. This is interesting since perceptual phenomena like preference for musically consonant intervals might be associated with roughness perception which has its perceptual basis in critical bands of the cochlea (Plomp & Levelt, 1965). In conjunction with earlier studies on rats (Borchgrevink, 1975) my data might give some indication that such phenomena are not based on culture but might be a result of how the cochlea processes sound. Place preference or two alternative forced choice experiments on animals could be useful to separate cultural from genetic factors when answering questions related to human music perception. If such experiments on animals were successful it may turn out that some aspects of human arts or aesthetics may not be purely cultural but may have been primed by how our sensory organs work. Another similarity between seals and humans was found: Although there was some variation in sensation levels at the edge of the deterrence ranges, values generally ranged from 60 to 70 dB. If one accepts the assumption that behavioural avoidance responses somehow reflect the onset of slight stress; then the mentioned sensation levels in seals are very similar to physiologically measured discomfort thresholds in humans (see Spreng, 1975).

The experiments described in chapter 5 elicited much stronger responses than those described in chapter 3. The test stimulus was designed to elicit the acoustic startle reflex, an oligo-synaptic reflex arc located in the brainstem that is elicited by loud short-rise time stimuli and leads to a sudden contraction of flexor muscles. The majority of the seals (n=5) showed extreme avoidance responses to the startle pulse leaving the pool and generally avoiding to approach the feeding station. The likelihood of the occurrence of flight responses and interruption of foraging behaviour increased over time, reaching 100% in the last trials. However, three animals showed only small avoidance responses that declined even further. A review of the startle literature and analysis of muscle contraction responses in the two groups of seals revealed that this may be due to differences in the animals hearing thresholds. This would mean that the test stimulus was not loud enough to elicit the reflex in all animals. Although the data shows that the flight responses are under voluntary control, startle seems play a role in the initiation of extreme avoidance behaviour. In some sense the behaviour of the two groups of seals followed a typical “all or nothing” principle, a phenomenon quite common in neurophysiology: if animals showed signs of the startle reflex they would always sensitise, however, if the reflex was not triggered they would habituate. This result was confirmed by a second experiment using a modified staircase procedure to measure startle thresholds in both groups of animals. In the group of animals that sensitised stimuli of about 160 dB re1µPa elicited startle responses while in the other three seals startle thresholds could not be determined due to limitations on the maximum output of the loudspeaker (and ethical concerns). If received levels are translated into sensation levels using published audiograms for the harbour seal (appendix 1) it becomes obvious that similarly loud stimuli elicit startle in seals and terrestrial mammals (80-85 dB above hearing threshold). The field trials showed that startle eliciting stimuli lead to larger-scale flight responses. Received levels at the edge of the zone within which no seals were observed (150-155 dB re 1µPa) were similar to the startle thresholds measured in the captive experiment. This gives further evidence for the important role of the startle threshold with respect to avoidance responses. In the captive experiment the startle stimulus was also paired with a substantially weaker pre-sound and animals quickly developed similarly aversive response to the pre sound stimulus. Initially non-aversive sounds can therefore gain aversive properties after just a few pairings with a startle pulse.

The first ever underwater playback experiment on grey seals calls showed that seals exhibit strong attraction responses to some calls, namely moans, rupes and rups. The results suggest that these calls might be used in mate attraction or as more general contact call e.g. around haulout sites. Growls and type 10 calls only caused occasional approach responses and might be used in close agonistic encounters between seals. Since calls cause strong attraction responses they might also have an additional function in order to establish underwater communication networks that could mediate haulout formation in a highly variable habitat e.g. on pack ice. When trying to interpret the function of grey seal call the biggest problem is the lack of information on the context in which underwater vocalisation are used. My playback study was primarily driven by the question whether playbacks of conspecific calls can elicit movement responses. However, if there had been information on how grey seals use underwater calls the choice of playback stimuli and questions could have been much better informed. Since grey seals do not seem to respond to the presence of human divers it may be possible to use a combination of underwater filming and a hydrophone array recordings to localise callers and collect data on how grey seals use underwater vocalisations. It would probably be even more enlightening (but also more expensive) to use sophisticated methods like a critter-cam (video system) attached to the seal or even better accelerometer tags that provide both fine-scale data on movement and vocalisations (as developed and successfully used by Johnson et al., 2004). The haulout initiation hypothesis could be tested further by conducting playback experiments in pack-ice breeding populations or by investigating calling behaviour (e.g. call exchange or matching events) and behaviour around a haulout site.

The experiment conducted around a fish farms (chapter 6) demonstrated differential responses in harbour porpoises and seals to short sound pulses designed to cause startle in seals. This demonstrates that absolute sound pressure levels are not a useful predictor for marine mammal responses to noise but sensation levels seem be important. The different behaviour of both species can be explained by the fact that the stimulus exceeded the startle threshold in seals in the vicinity of the fish farm. In contrast the stimulus would only exceed the assumed startle threshold of a porpoise if an animal was closer than 3-4 m from the transducer (the closest observed approach of a porpoise was ever seen from the transducer was 8 m). Sound field measurements confirmed that the edge of the area from which seals were excluded coincides roughly with the measured startle threshold from the captive experiments. In conclusion physiological predictions should be considered when addressing conservation related issue like specifically targeting certain predators.

In conclusion all experiments showed that although factors governing marine mammal responses to sound are complex there are certain rules and responses might be more predictable than some authors have suggested (Southall et al., 2008). In fact some of the difficulties in explaining variability in behaviour in previous studies might in part have resulted from the fact that the data was not cross-checked against the physiological literature on standard models like rodents or humans (e.g. startle reflex or discomfort threshold). In contrast the argument that “marine mammals are different” is sometimes heard. However, the data from all my experiments on seals and porpoises are consistent with several concepts derived from human data. For example, sensation levels seem to be associated with perceived loudness in marine mammals (at least within the most sensitive hearing range). This finding is almost surprising given that in some cases published audiograms for closely related species had to be used (e.g. harbour versus grey seals) which could have introduced some error. It was however also found that sound characteristics other than sound pressure influence responses in seals e.g. similar to humans high roughness of a sound leads to higher aversiveness. In addition motivation has been shown to be a crucial factor and might have counter-intuitive effects e.g. animals might stay in an area tolerating exposure to high noise even though there is currently no food available if the location is known as a potential foraging hotspot (see results in chapter 3). Apart from these factors I found that certain physiological thresholds are crucial predictors for behavioural responses to sound. This means that behavioural responses do not always increase gradually with increasing received level but sometimes follow more an “all or nothing principle”. The most obvious and best understood example is the startle threshold which was found to be associated with levels exceeding the hearing threshold by 80-85 dB. The startle threshold turned out to be a good predictor for extreme avoidance behaviour and sensitisation to sound. While stimuli below the startle threshold had some aversive effect it seems that if the threshold is exceeded the response changes categorically (see chapter 5). Another example for a physiological threshold that bears behavioural relevance might be the onset of discomfort which may manifest itself as a moderate avoidance response. Although the neuro-physiological basis for discomfort is much more dubious compared to startle, if the onset of mild avoidance behaviour reflects discomfort (see Kastelein et al., 2005) then the discomfort threshold would occur at similar sensation levels as in humans (sensation level of 60-70 dB).

Development of an efficient and target-specific acoustic deterrence system for fish farms and fisheries

One objective of all described experiments was to design a more effective ADD that deters seals from fish farms but has no effect on other wildlife (particularly on *odontocetes*). The playback using biological sound was rather unsuccessful: None of the tested grey seal calls caused a deterrence effect but playbacks attracted seals to the loudspeaker. While grey seal calls are therefore unsuitable for acoustic deterrence they may be used to trap and relocate “rogue” seals. The paradigm applied for all tested non-biological sounds (startle and artificial sounds) was to shift the frequency band down in order to create stimuli that cause higher sensation levels

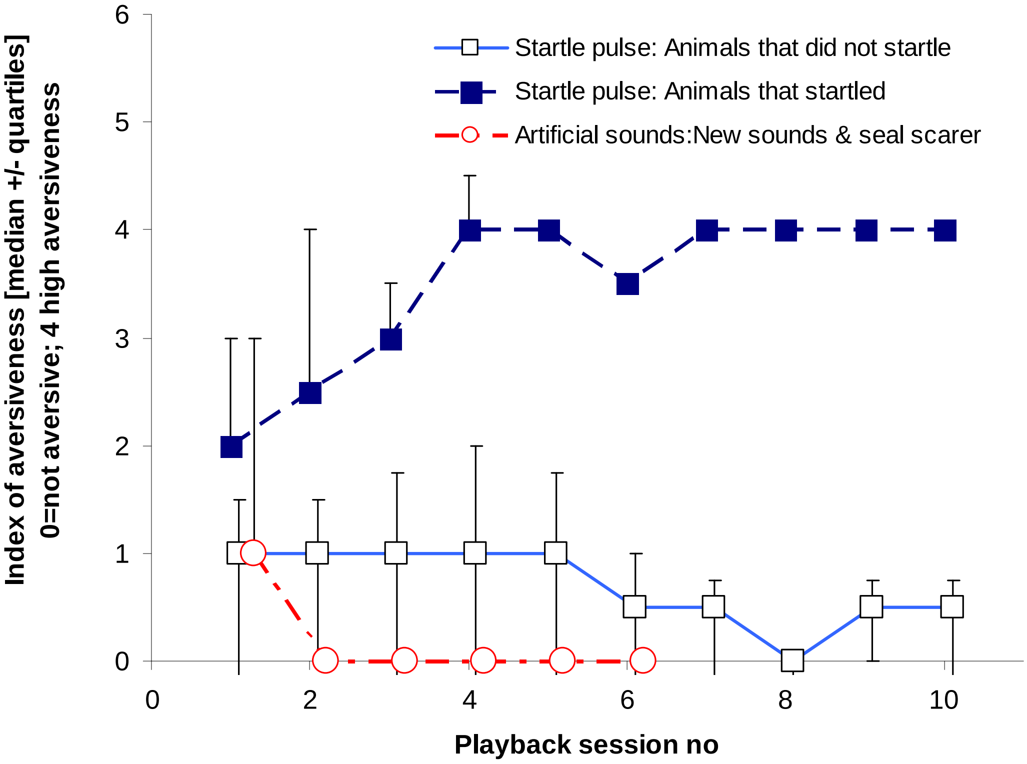

Figure 1: Effectiveness of the sounds tested sound from chapter 2 and 4 in deterring captive seals from a feeding station. The figure shows that a short pulse that exceeds the startle threshold is the most promising approach and seems to replace habituation by sensitisation.

in seals than in *odontocetes*. In comparison to current seal scarers this would reduce sensation levels for *odontocetes* by 30-40dB (see chapter 2). In terms of efficiency, the first study (chapter 3) showed that seals avoided the high-duty cycle novel sounds and the sounds of commercial seal scarers on the first exposure but they habituated quickly and returned to their known feeding station next to the underwater speaker. However, the brief but high source level (171 dB re 1µPa @ 1 m) sound pulses designed to elicit a startle response (chapter 5) did have the opposite effect on the majority of the seals. All animals for which there was evidence that the startle reflex was elicited by the sound pulse showed flight responses and sensitized. Animals for which the stimulus was not loud enough to elicit the reflex habituated. A direct comparison can shed light on the differences in efficiency between the different sound types. Figure 1 shows the average index of aversiveness (for a definition see chapter 3) for the startle experiment and the experiment that tested the commercial ADD and artificial new sounds over all playback sessions. It is obvious that the startle pulse was most effective in deterring animals from a foraging spot while the higher duty cycle artificial sounds (e.g. current seal scarers) were rather ineffective. This was also confirmed by the fact that startle stimulus was among the most effective in the field trials around haulout sites. Figure 1 also shows that the startle pulse caused sensitisation only if it actually exceeded the startle threshold in a certain individual (otherwise the animals will still habituate). As I discussed in chapter 5 data on rodents showed a strong correlation between hearing and startle thresholds in rodents. If this is the case in seals as well then it would be important for a startle-based acoustic deterrence system to dteremine a source level that results in received levels that are loud enough to exceed the startle threshold even in animals with slightly compromised hearing. However, received should not be too high to avoid inflicting TTS which in turn would lead to an undesirable elevation of the startle threshold. Given that sound pulses sufficient to cause startle can be very short and would only have to be produced at a very low duty cycle this is possible. The startle threshold in seals was about 160 dB re 1µPa and using equal energy criteria and TTS data from Kastak et *al.* (2005), TTS in response to a single 200ms sound pulse would only occur if sound pressure level of 190 dB re 1 µPa were reached. So there is at least a 30 dB difference between onset of startle and TTS in a healthy animal. The general feasibility of applying the startle paradigm in a seal scarer was also shown by the field trials based on a fish farm. The prototype ADD managed to deter seals from the farm for the whole 2 month test period but it did not affect the distribution of harbour porpoises in the area. Porpoises were seen as close as 8m from the operating transducer. It also did not affect the number of minke whales, however, these animals were never seen closer than 1km from the transducer even during control sessions. Based on the literature, salmon cannot hear this startle sound but fish with more sensitive hearing could be affected within 100 m of the speaker (see discussion chapter 6).The fish farm experiment shows that it is possible to completely mitigate impact of seal scarers on *odontocetes* although potential effects of low-frequency hearing specialists should be investigated. Devices using this novel stimulus should therefore be tested long-term on fish farms since my lab studies show that this method holds some promise to be more effective than current ADDs. The field study (chapter 6) showed that this method would not have detrimental effects on the distribution of toothed whales (e.g. harbour porpoises). However, responses on low-frequency hearing specialists (e.g. herring and ballen whales) should be investigated.

I believe that companies aiming to develop successful acoustic deterrence devices and researchers trying to draw conclusions from empirical studies should have a thorough look at the neuro-physiological literature. In many cases seemingly contradictory findings may simply originate from basic physiological paradigms like reflex elicitation thresholds in combination with certain sound propagation characteristics. It seems that the development of an effective acoustic deterrence system was hampered by the fact that mostly devices made available by companies were tested. However, companies seem to come up with problematic concepts without any clear evidence in support. One example is the patent filed for the Airmar seal scarer (see Jeffers, 1995) which suggests that the device causes pain while not exposing the animal to received levels that cause hearing damage. This was based on the assumption that animals moving around in the sound field would always avoid areas where the pain threshold is exceeded. The patent also assumes that animal would not habituate, however, the data from experiment 1 in chapter 3 shows that they habituate quickly to high duty-cycle sounds. It is also assumed that hearing damage does not occur below the pain threshold which as I have argued in chapter 2 is also not true. I suggest that making use of the acoustic startle response might be the key for successful acoustic deterrence with the potential to replace habituation by sensitisation. Such a paradigm requires good physiological understanding of the underlying mechanisms which should for example be investigated in cetaceans. The startle paradigm offers the opportunity to dramatically reduce noise pollution due to the low required duty cycle. My prototype ADD operated at a very low duty cycle of 0.08 %. However, a responsive mode design, meaning that sound is only produced when an animal approaches could reduce the duty cycle even further. Furthermore, startle stimuli can be designed to specifically target a certain taxon by exploiting differences in the species hearing threshold. A startle pulse needs to be designed to exceed the startle threshold in the target group of animals but not in another group within a certain area. The feasibility of this was demonstrated in the fish farm experiment (there might obviously be limits on that depending on which species are abundant in the area). In conclusion ADDs based on startle might be useful for many applications e.g. reduction of *odontocete* depredation on trawls or long-lines or excluding seals from salmon rivers, fish farms or fisheries.

The startle reflex in marine mammals: Biological function and potential application as a research tool

From an evolutionary point of view my data supports the hypothesis that the main function of the startle reflex lies in increasing an animal's propensity for flight by bringing the body into a state of "alertness". Seals showed a similar but delayed sensitisation effect to the pre-sound and behaved similar to rats tested in fear conditioning paradigms with electric foot shocks as an unconditioned stimulus. This might indicate that the startle reflex leads to physiological changes that induce fear which would influence the probability of flight. I believe that this hypothesis should be tested thoroughly. This could be done by measuring parameters like hormonal changes, galvanic skin responses, heart rate and behaviour in conjunction with startle amplitudes. Startle amplitudes can be quantified by means of an accelerometer sensor or electro-myographic methods monitoring muscle activity above baseline.

It would also be interesting to investigate the startle response in *odontocetes* since they use high source level but short (50-200 µs) clicks for echolocation (Au, 1993). *Odontocetes* have evolved mechanisms for regulating their hearing sensitivity differentially in responses to their own outgoing clicks and projected external clicks depending on the presence of an acoustic target (Supin et al., 2006). It is therefore possible that in spite of the fact that the startle reflex is similar in many mammalian species, *odontocetes* show some interesting modifications on a neuronal level. Since it is known that startle amplitude in rodents decreases rapidly if signals are shorter than 1ms (Marsh et al., 1973) it is however also possible that echolocation clicks are less likely to elicit startle anyway.

In highly restrained lab settings neither humans nor animals show overtly high behavioural follow up responses after being startled and the use of sensitive methods to quantify startle amplitudes is standard practice in clinical situations and for research applications (Blumenthal et al., 2005). It should therefore be mentioned that the startle reflex holds great potential with respect to studies on audiometry and sound discrimination. Young & Fechter (1983) showed that pre pulse-inhibition paradigms can be used for audiometry. This is done by monitoring temporary recovery of inhibited startle amplitudes when sub-hearing threshold pre pulses are presented. This might prove an interesting alternative to auditory-brainstem response (ABR) measurements in marine mammals. Also, similar paradigms have been used in rats to investigate discrimination of speech sounds (Floody & Kilgard, 2007). If done conventionally this study would have required very time-intensive training using operant conditioning. These finding should be of interest to marine mammals researchers e.g. when studying captive animals that either difficult to access (e.g. polar bear). Startle based audiometry could also useful for species in which measurements of auditory evoked potentials with surface electrodes (AEPs) are difficult to obtain underwater (e.g. seals, pola bears).

Could the startle reflex be relevant for beaked whale mass strandings in response to mid-frequency military sonar?

Several authors reported a link between naval exercises and beaked whale mass strandings in different regions of the world (Frantzis, 1998; Anonymous, 2005; Fernandez et al., 2005; Jepson et al., 2003; Simmonds & Lopezjurado, 1991). In most documented cases these naval exercises involved the use of mid-frequency active (MFA) sonar systems emitting frequencies between 2 kHz and 15 kHz at source levels (rms) as high as 235 dB re 1µPa (see Cox et al., 2006). However, it seems that mass stranding are sometimes also associated with other sound sources e.g. air guns or sub-bottom profilers (Anonymous, 2003; Anonymous, 2004). Jepson et *al.* (2003) found that stranded beaked whales had gas bubble lesions and suggested that exposure to sonar might have caused changes in diving behaviour e.g. interruption of deep foraging dives which could have led to decompression sickness (DCS). Recent modelling suggested that DCS was more likely to occur as a result of an extended series of shallow dives e.g. during flight (Zimmer & Tyack, 2007). One interesting suggestion how such a response could be mediated is that the sonar resembles killer whale calls possibly creating some kind of “super-stimulus” that causes a strong predator avoidance response (Zimmer & Tyack, 2007). Since strong long-distance avoidance behaviour in cetaceans has also been shown in response to artificial sounds that do not resemble killer whale calls (Johnston, 2002; Olesiuk et al., 2002) I suggest that a startle response could lead to a similar response. This is underlined by the fact that it is likely that the startle reflex itself has evolved in the context of predator avoidance (Pilz & Schnitzler, 1996). It could, therefore, play a role in conjunction with the predator recognition scenario suggested by Zimmer & Tyack (2007) or even work on its own when sounds do not resemble predators. As described in the discussion of chapter 5 the data from my experiments give evidence for flight responses being tightly associated with the startle reflex since overtly high avoidance responses were only caused in seals that startled. In addition the fact that seals behaved very similar to rodents in “fear conditioning experiments” suggests that the startle reflex is associated with physiological changes inducing fear. D’Spain (2006) reported that some stranding events were associated with the presence of an acoustic waveguide leading to low transmission losses and even more importantly the occurrence of unusual transient pulses with rapid onset or decay time, stimuli suitable to elicit strong startle responses (see Fleshler, 1965). If startle plays a role then two factors are likely to be important. 1.) Received levels at the animals head would have to exceed the startle threshold (all or nothing principle) 2.) The amount by which the received levels exceeds the startle threshold would determine the startle amplitude which might in turn determine the strength of the avoidance response (see Pilz et al., 1987). My data gives evidence for a relationship between startle threshold and occurrence of flight responses, however, a correlation between startle amplitude and strength of avoidance behaviour has not been investigated. A suggestion that this correlation could exist in marine mammals comes from the fact that two of the seals that had higher startle thresholds sensitised slightly later. If a certain startle magnitude is necessary to elicit an overtly strong avoidance response then this could explain why beaked whales only strand in response to some pulsed noise sources or scenarios. Signals from fish finders and some multi-beam sonars are often less than 1ms long (Richardson et al., 1995 and own findings) and would therefore only cause very small startle amplitudes (see Marsh et al., 1973). Continuous background noise is known to elevate startle amplitudes (Hoffman & Ison, 1980; Hoffman & Fleshler, 1963b). Therefore, noise created by the presence of many ships during a naval exercise could lead to higher startle amplitudes and possibly stronger avoidance behaviour. If multi-path propagation leads to the arrival of two consecutive transient pulses (less than 10 ms apart) one above and one below the startle threshold then this would lead to a phenomenon called pre-pulse facilitation (PPF) resulting in an increased startle response (Ison et al., 1973; Hoffman & Ison, 1980). While these examples are currently only speculative scenarios, they need further consideration. Startle thresholds and reflex modification mechanisms could be investigated using a combination of electro-myographic methods (quantifying startle) and auditory brainstem responses (quantifying hearing threshold; e.g. in captive *odontocetes*). Measuring responses to loud pulsed sounds versus predator calls and sonar might prove a useful method to try to quantify the contribution of different mechanisms to strong avoidance responses in marine mammals. In that context an increase in rise-time may also be worth investigating if it could be used to mitigate impact of anthropogenic noise pulses by avoiding startle responses.

Marine mammal noise exposure criteria

Southall et al. (2008) published a comprehensive evaluation of the current literature on marine mammal behavioural and physiological responses in an attempt to define safe exposure criteria for all taxa. The document is an impressive review of the current literature containing contributions by the leadings experts; it is however surprising that some of the published values for behavioural disturbance are very high. This has in part to do with the fact that for example for exposure to single pulses onset of temporary threshold shift was used to define “behavioural disturbance”. Southall et al.’s (2008) approach might also not be very conservative since animals in the reviewed studies had not always been tested with stimuli that fell in their most sensitive hearing range (e.g. data used for pinnipeds exposed to multiple pulses). Thus, if received levels are not translated into sensation levels derived exposure criteria might be too high. In the following section I will suggest alternative values based on the observed behaviour in my experiments. Given the evidence for the importance of sensation levels in the context of behavioural disturbance (see e.g. chapter 3 and 5) I will additionally express sound pressure levels in this metric.

It has been suggested that sound exposure levels (SEL) are a useful metric to define dose-response relationships for marine mammals (Southall et al., 2008). While there is good evidence for this with respect to phenomena like auditory fatigue (TTS) or hearing damage, there is no evidence that this is the case for behavioural responses. While it is possible that SELs are useful for predicting responses to continuous noise of different durations, my data generally challenge their usefulness. The captive experiment in chapter 3 involved exposure to a sound pressure level of 147 dB re 1 µPa for 24s within the 1min experimental period. The SEL was therefore approximately 161dB re 1µPa^2-s. In the startle experiment (chapter 5, exp. 1) a maximum of two 200ms pulses were presented at a source level of 170-171 dB re 1µPa within a 3min experimental period. The average exposure time per minute was therefore 133 ms resulting in a sound exposure level of 162 dB re 1µPa^2-s. These calculations show that if sound exposure is compared over the same time interval the SELs in both experiments were almost identical, however, as fig 1 shows the behavioural responses were fundamentally different. I therefore believe that SELs are not a good predictor for behavioural responses but noise exposure criteria should be based on biologically meaningful thresholds like the startle threshold. However, since the advantages and disadvantages of SELs and sensation levels are not clear for all types of noise, I incorporated both in the following considerations. Sound exposure levels were calculated by equation 1 (see also chapter 2):

$SEL=SPL+ 10*\log (t)$ (Equation 1)

with t being exposure time in seconds and SPL the sound pressure level re a specific reference value.

I suggest that using a sound exposure level (SEL) referenced to the hearing threshold rather than 1µPa would be a useful approach. This is particularly true for behavioural responses where sensation levels seem to play a major role (see chapter 3 and 6). Such a “sound exposure level-sensation level” (SEL-sensation level) is mathematically correct since the sound pressure level term (SPL) in equation 1 is based on a reference value. The reference value is usually set to 1µPa, however, one could justifiably use the hearing threshold instead. This is a common procedure for applications in air where 20 µPa are used as a reference value which is the human hearing threshold at 2.5 kHz. For comparative purposes across tested subjects, such a calculation had also been provided by Kastak et al. (2005).

In the following section “SEL” (sound exposure level) are referenced to 1µPa. However, “SEL-sensation levels” (sound exposure level-sensation levels) are referenced to the hearing threshold of the tested species. Therefore, using this criterion for another species requires inserting the respective hearing threshold in Pa. The following noise exposure criteria are based on my data and the approaches used to derive them should be considered as mere suggestions open to academic debate.

Continuous noise

The field trials tested sounds from current seals scarers and a variety of artificial high duty-cycle sounds (all sound were played at 50% duty cycle). The average received level that caused a significant reduction in seal numbers for the 4 sounds that were tested at least 10 times was 135 to 140 dB re 1µPa. The average avoidance threshold of the two control sounds and two new sounds as calculated from table 3 in chapter 3 expressed as sensation level was 66 dB re hearing threshold. The maximum continuous exposure time in the experiment was 10s. Therefore, using equation 1 to calculate a sound exposure level (SEL) referenced to the hearing threshold would require to adjust by 10*log (10s). This would result in a “sound exposure level-sensation level” of 76 dB re (hearing threshold in Pa)^2-s. Alternatively, one could take the sound exposure time over the whole 5 min observation period into account (150s); then “SEL–sensation level” would be 88 dB re (hearing threshold in Pa)^2-s.

As we have seen in chapters 2 and 3, one could argue arguments that discomfort thresholds expressed in sensation levels are the same across mammalian taxa. Thus, for a harbour porpoise that hears a 16 kHz tone, “SEL-sensation levels” might also be 76 dB and 88 dB re (hearing threshold in Pa)^2-s. So, inserting the hearing threshold in the term would result in an SEL-sensation level of only 120 dB 1µPa^{2}s and 132 dB 1µPa^{2}s respectively (hearing threshold: 44 dB re 1 microPa according to Anderson, 1970).

Using equation 1 to calculate a noise exposure criterion directly from the measured received levels at the edge of the deterrence range (140 dB re 1 µPa) would result in a noise exposure criterion of SEL of 150 dB re 1µPa^{2}s.

To summarize the suggested criteria for continuous noise would be:

Pinnipeds:
SPL (rms) for: 135-140 dB re 1µP (sound tested ranged from 500Hz to 20 kHz)

General criteria:
Sensation level: 66 dB re hearing threshold
SEL-sensation level: 76 dB or 88 dB re (hearing threshold in Pa)^2 s

Pulsed noise

The behavioural responses observed in the animals that sensitised in the captive experiment (exp. 1 chapter 5) would fall in the second most severe category (level 8 on the scale specificially designed for captive experiments according to Southall et al. 2008). Therefore, if one aims to define noise exposure criteria for short rise-time (<15 ms) pulses that exceed the startle threshold in order to protect animals from exhibiting strong avoidance responses, then received levels should not exceed the startle threshold. However, as shown in the field experiment (exp. 3 chapter 5) pulsed noise at lower received levels (145 dB re 1µPa) below the startle threshold still caused moderate avoidance behaviour (level 6 response for experiments in the wild, see Southall et al. 2008).

The startle threshold for seals exposed to a 1 kHz pure tone determined in chapter 5 was 160 dB re 1µPa. The received level for pulses with short rise-times at the edge of the deterrence ranfe in the field in chapter 5 was 150-155 dB re 1µPa (based on the closest ever observed approach of a seal during sound exposure). In conclusion, the startle threshold data would suggest a noise exposure criterion of SPL of 150-160 dB re 1µPa. As mentioned in chapter 5 in terms of sensation level this would be 85 dB above the hearing threshold.

The maximum sound exposure level in the captive startle experiment (exp 1; chapter 5) calculated over the 3 min observation period was 167 dB re 1µPa^2-s dB. This would however be an upper limit; the actual criterion might have to be lower since the level for onset of extreme avoidance was not determined in the experiment. Assuming a hearing threshold of 75 dB re 1µPa at 900Hz (extrapolation from values given in appendix 1) the "SEL-sensation level" would be 92 dB re (hearing threshold in Pa)^2-s. If *odontocetes* have similar startle thresholds re hearing threshold then the "SEL-sensation level" of 92 dB re (hearing threshold in Pa)^2-s could also be used to calculate a exposure criterion for this taxon. The criterion for a harbour porpoise that hears a 16 kHz pulse would result in an SEL of only 136 dB 1µPa^2-s. This is largely due to the fact that the hearing threshold at 16 kHz is very low (44 dB re 1 µPa according to Anderson, 1970).

Data from the field trials (exp 3, chapter 5) based on the estimated maximum deterrence range shows that received levels of 145 dB re 1 µPa still reduced the number of animals sighted significantly. Assuming the hearing threshold to be 75 dB re 1µPa at 900Hz (extrapolation from values given appendix 1) the sensation level would be 75 dB re 1µPa. The overall sound exposure time over the 5min was 2.4s (12 x 200ms pulses). Using equation 1 would therefore result in a "SEL-sensation level" of 79 dB re (hearing threshold in Pa)^{2}s.

The given values would indicate that current noise exposure criteria for behavioural responses should be lowered by 30-40dB. As long as the startle threshold in *odontocetes* has not been determined this should also be done for toothed whales using the seal data. In my opinion, such an approach is more appropriate than using TTS as a criterion for behavioural disturbance.

To summarize the suggested noise exposure criteria for single and multiple pulses would be:

Pinnipeds:
SPL (startle threshold, level 8 on severity scale):
155-160 dB re µPa (rms)/164 dB re µPa (peak) at 1 kHz (pulses with short rise-time)

SPL (moderate avoidance, level 6): 145 dB re 1 µPa (see also chapter 5;any pulse, peak frequency of 900 Hz)

Generic criteria:

Sensation level (severity level 8): 80-85 dB re hearing threshold
SEL-sensation leve (severity level 6): 79 dB re (hearing threshold in Pa)^2-s

My data also provide information on noise exposure criteria with respect to repeated exposure to pulses. Figure 2 shows a dose-response relationship curve for the number of startle pulses presented and the probability of occurrence of fast flight responses followed by jumps out of the pool for the five animals that startled. Note that this is different from the previously presented plots due to the responsive mode protocol of the playback (see chapter 5). A logistic regression model fitted to the data was highly significant ($F_{2,23}$=136.903, p<0.001) and explained 92 % of the variance. Using the equation from the regression in fig 2 results in the prediction that exposure to 8-9 pulses would cause flight responses in 80% of animals. Given that the seals seemed motivated to stay close to the loudspeaker (food presentation), this again supports the call for a more conservative approach to sound exposure criteria for marine mammals for pulsed noise with short rise-times.

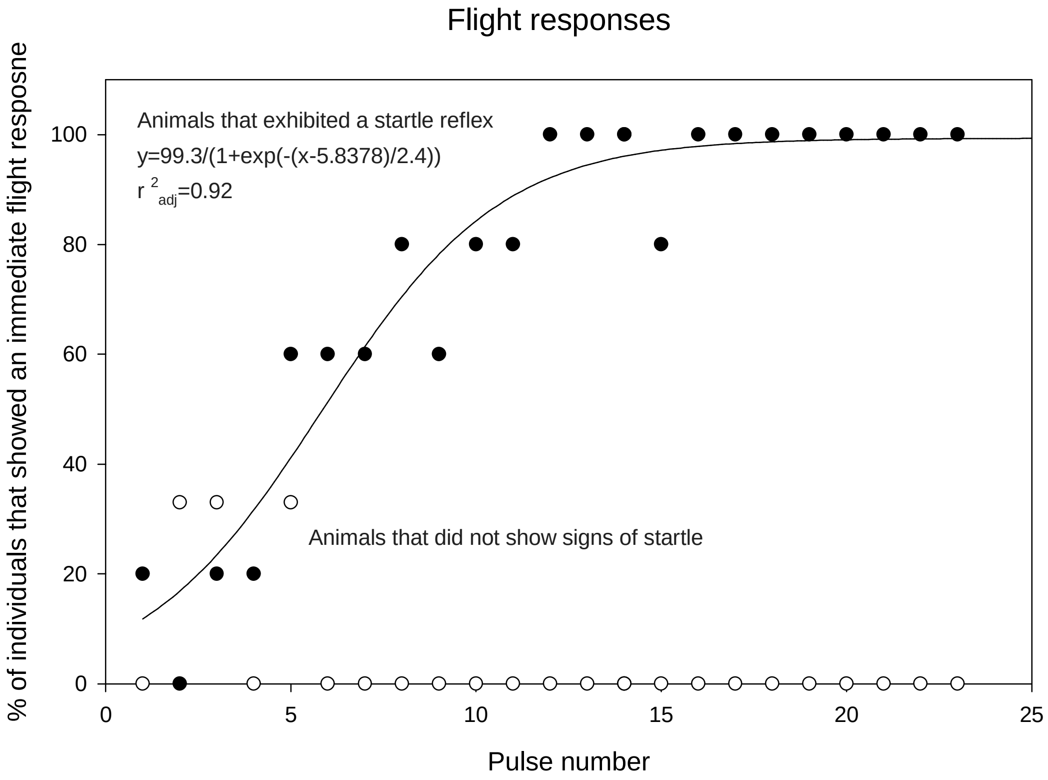

Figure 2: The figure shows the percentage of individuals that showed a flight response in the first startle experiment versus pulse number. This is shown for both groups of seals separately. The logistic regression shows that if a stimulus is loud enough to startle an animal then 80% of individuals will respond extremely after just 8-9 presentations.

Finally, one should appreciate the possibility that responses to pulsed noise with short rise-times might not be determined by the overall amount of energy but the number of individual pulses and whether or not a stimulus exceeds the startle threshold. Therefore, the number of elicited startle responses may be good predictor. The dose-response relationship in figure 2 would suggest that a conservative criterion would be to avoid exposure to more than 8 short rise-time pulses that exceed the hearing threshold by more than 85 dB within a 24 period. In conclusion more research should be done investigating the startle reflex and behavioural follow-up responses in marine mammals (particularly in odontocetes)

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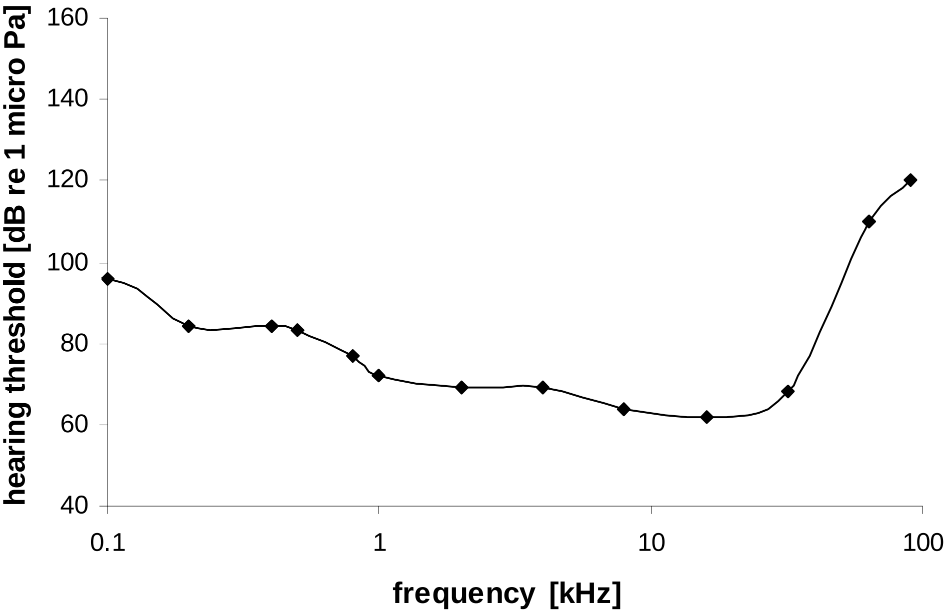
Appendix 1: Composite audiogram for harbour seals



Studies from which values at a given frequency where averaged	**Frequency (kHz)**	**Threshold (dB re µPa)**
Mohl (1968): M Kastak & Schusterman (1998): KS Terhune (1988): T		
KS	0.01	102
KS	0.1	96
KS	0.2	84
KS	0.4	84
extrapolated from KS	0.5	83
KS	0.8	77
KS&T	1	72
extrapolated from KS, T	2	69
extrapolated from KS, T	4	69
KS, M, T	8	64
M,T	16	62
M, T	32	68
M, T	64	110
M, T	90	120

The audiogram includes available behavioural data for harbour seals. The tables shows the averaged or direct values obtained from studies by Terhune (1988), Mohl (1968) and Kastak & Schusterman (1998)

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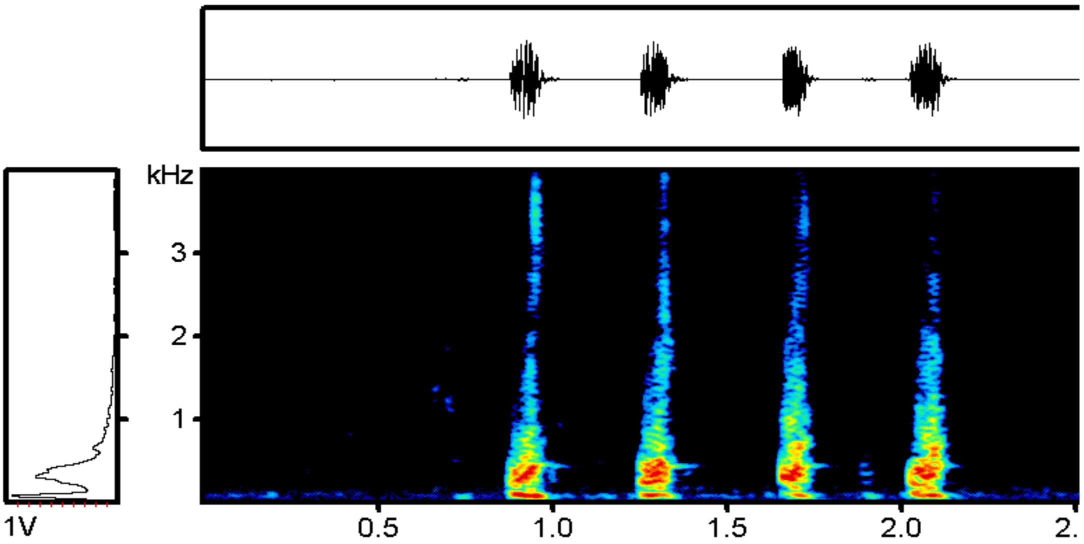
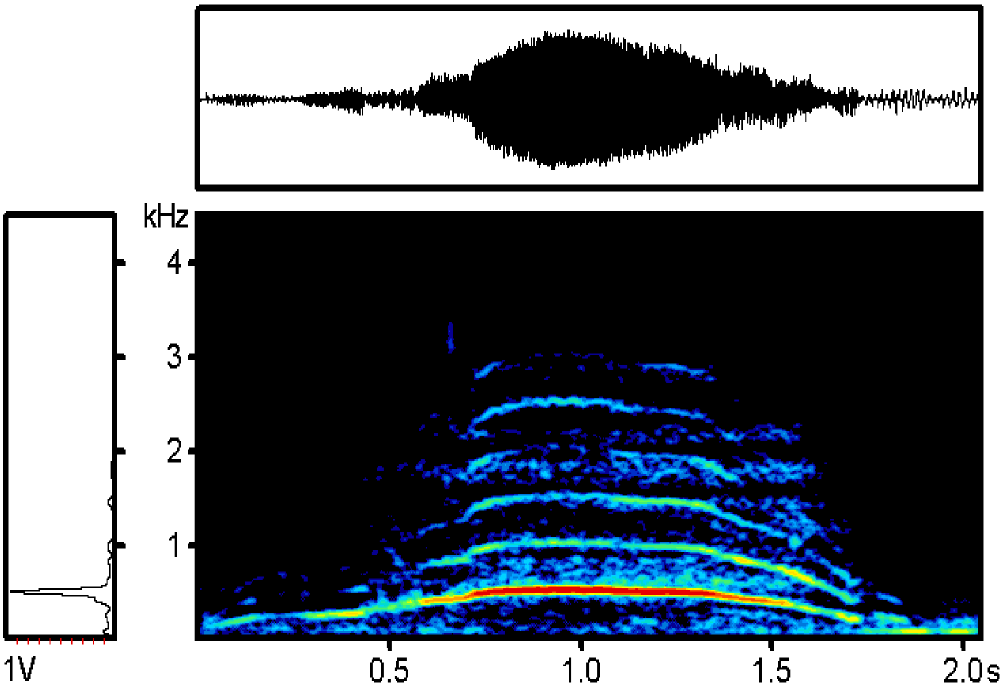
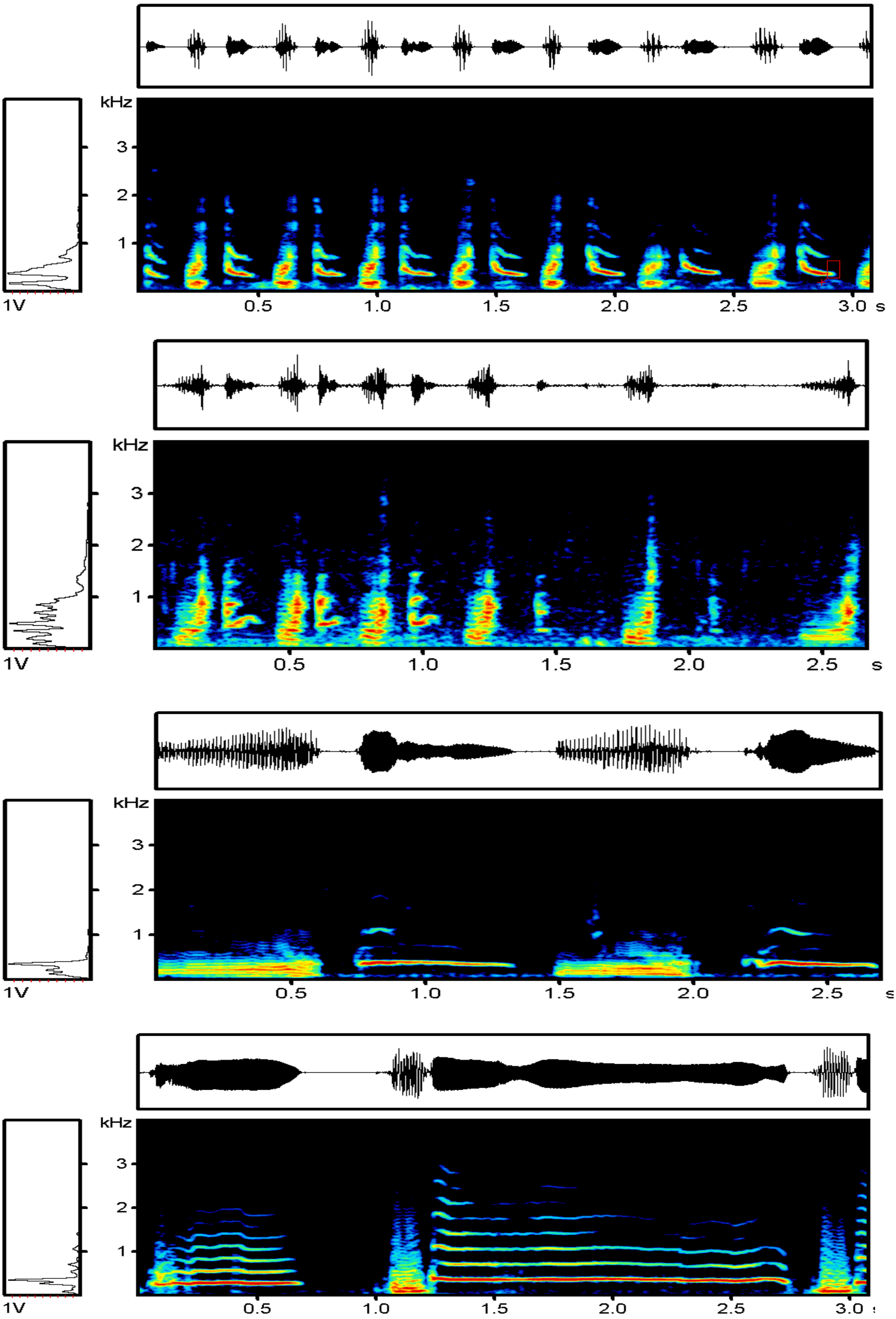
Appendix 2



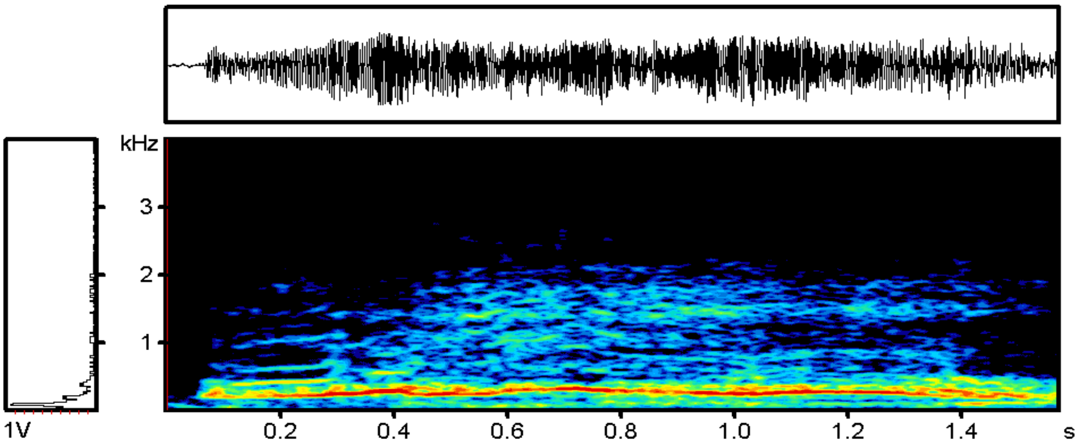
Rup
Rup, Type 1 (1A) (Mc Culloch 1999)



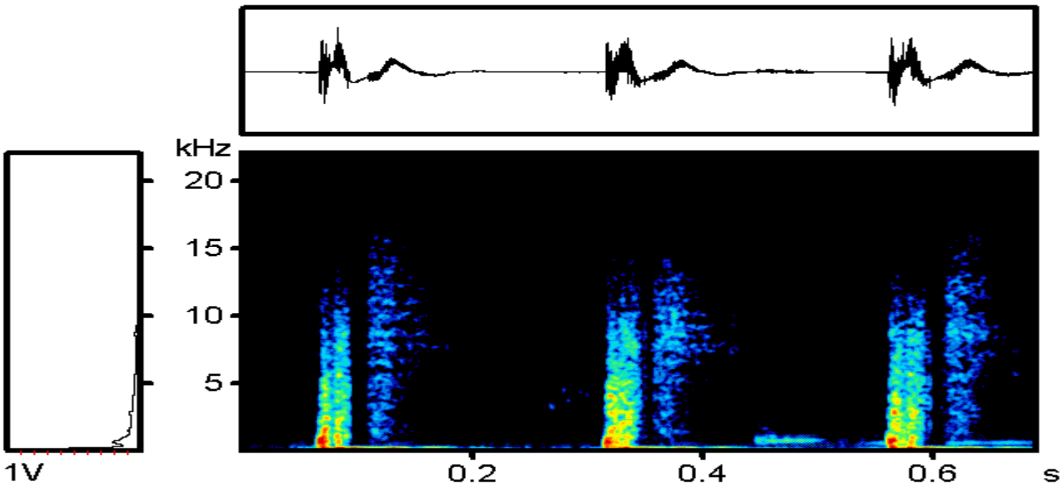
Moan
Moan, type 7 (Mc Culloch 1999)



Rupes: The most common version of this call is shown in the upper panel.
Type 5 (McCulloch 1999).



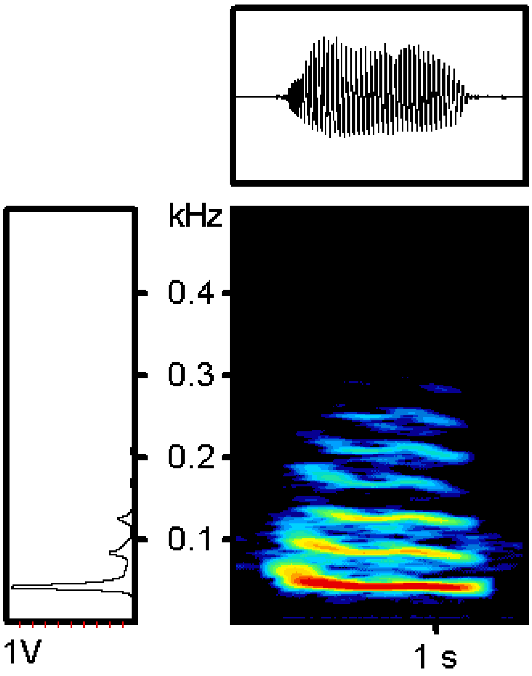
"Growl"
Type 9 (Mc Culloch 1999)

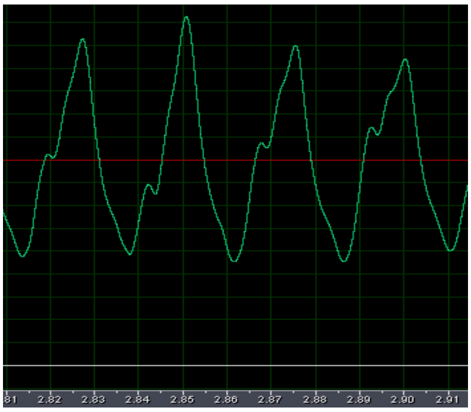


Knocks
Type6 (2H) (Mc Culloch 1999)

Type 10

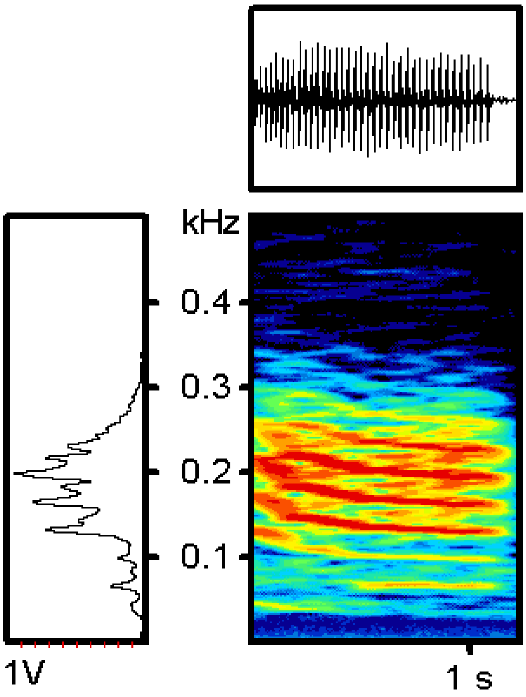
Type 10 (McCulloch et al. 1999)





Type 10 (2K)

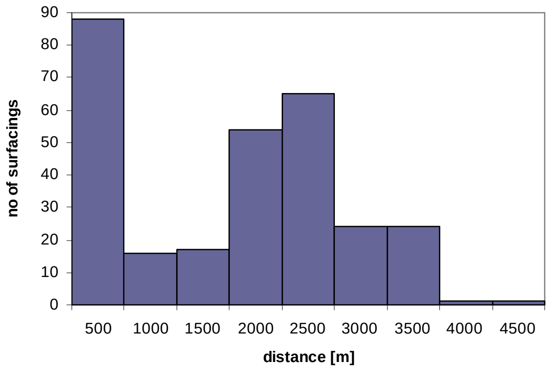
Type 10: (McCulloch et al. 1999)



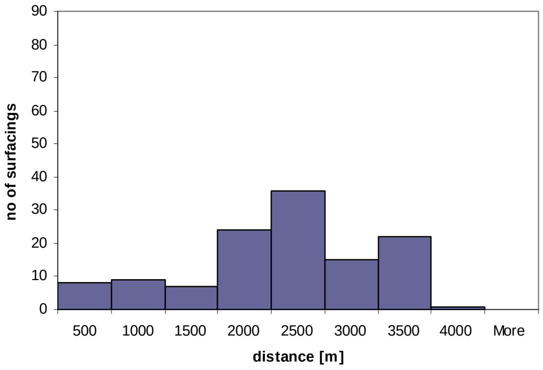
Appendix 3

Harbour porpoise

All sightings

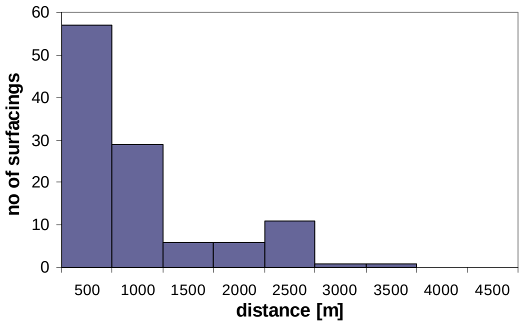


Control only



Seals

All sightings



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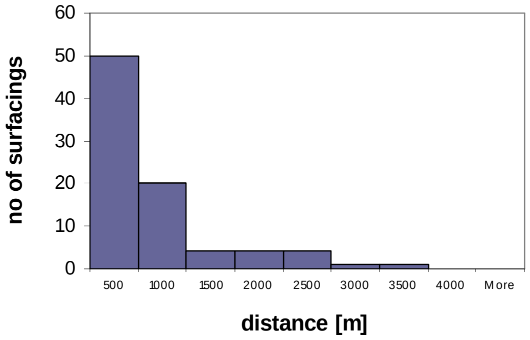
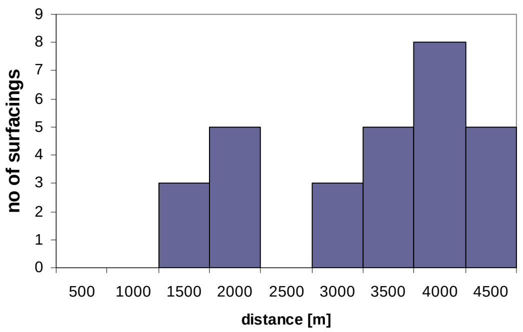


Minke whales

All sightings



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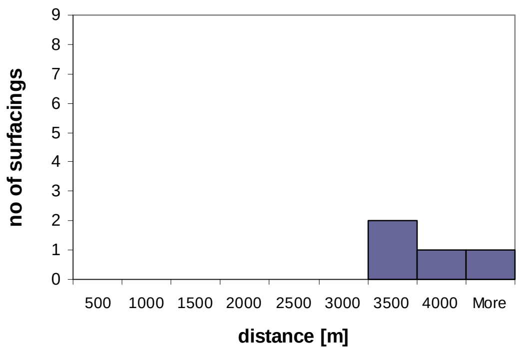

Appendix 4: Glossary of acoustic and psycho-physiological units

Auditory brainstem response measurements (ABR): Method of measuring acoustically evoked electric potentials with surface or thin needle electrodes in animals or humans. This method can be used to measure audiograms and other parameters related to sound perception.

Auditory evoked potential (AEP): A neuronal, electric potential that is evoked by an acoustic event; see auditory brainstem response measurements;

Auditory threshold: see hearing threshold

Audiogram: An audiogram displays the hearing threshold as a function of frequency. A lower sound pressure level value on an audiogram display reflects a low hearing threshold at a given frequency and hence a high auditory sensitivity (this means that even a very weak sound would still be audible to the animal). Audiograms in mammals are typically U-shaped reflecting the fact that hearing sensitivity declines towards the edge of the hearing range.

Hair cell: Sensory cells that act as an electro-mechanical transducer in the inner ear of vertebrates and in the lateral line systems of fish and amphibians. Damage to the hair cells usually results in a loss of hearing sensitivity (deafness).

Hearing threshold: The received level in the vicinity of the ear that is just audible to an animal/human. This must be considered an empirical term e.g. hearing thresholds can be defined as a 50 % or 75 % response threshold. Hearing thresholds depend on the frequency of the sounds and can vary strongly across species.

Loudness: Psychophysical unit to measure perceived magnitude of an acoustic stimulus in humans. Loudness is not equivalent to the SPL or the decibel scale but can best be modelled by a potential function. In humans loudness is measured in “sone” using psychophysical procedures.

Noise : A sound with a random waveform that contains energy across a broad range of frequencies. White noise has energy equally distributed across all frequencies.

The term is also used to refer to loud, unwanted and often annoying sound. Similarly, noise exposure criteria usually refer to certain threshold values below which a certain unwanted response (e.g. avoidance or hearing damage) is unlikely to occur.

Permanent threshold shift (PTS): Permanent, non-recoverable rise of the hearing threshold e.g. as a result of exposure to loud sounds or exposure for extended amount of time. PTS results in lower hearing sensitivity (meaning that a sound needs to be louder to be still audible). Strong PTS would manifest itself as deafness. PTS is a function of both, exposure time and sound pressure level. PTS is associated with the death of hair cells in the inner ear.

Peak-to peak (p-p) sound pressure:

The p-p sound pressure is difference between the maximum positive and negative measured sound pressure of a waveform. P-P sound pressure levels are useful to describe transient acoustic events where the rms-sound pressure value could potentially underestimate the risk of acoustic trauma.

Pulse: relative term used to refer to a short, non-continuous acoustic event

Received level (RL):

Measured sound pressure level at a given distance from the source. The received is always lower than the source level.

Rise-time: Time delay between the onset of an acoustic signal and point when it reaches its maximum amplitude or a pre-defined percentage of this amplitude.

Root mean square (RMS) sound pressure:

Square-root of the mean squared sound pressure of a waveform. The unit is generally used to describe the amplitude of continuous waveforms.

Sensation level:

Sound pressure level by which a stimulus exceeds an individuals hearing threshold. Equal sensation levels can be expected to roughly cause similar loudness perception. Using sensation levels in calculations can be expected to be comparable to A-weighting procedures used in humans (dBA).

Source level (SL)

Sound pressure level at 1m distance from the source

Sound pressure level (in decibel/dB): The sound pressure level SPL is defined as

SPL (in dB) =20*$\log_{10}$ (sound pressure/reference sound pressure)

The reference pressure in water is usually 1µPa while a reference value of 20µPa is used in air (which is the human hearing threshold at 2.5 kHz). The logarithmic scale of the SPL is useful to display the big dynamic range of an auditory system within a convenient range of values. The decibel scale also roughly models human perception of loudness. Adding 10dB to a given sound pressure level approximates a doubling of perceived loudness. SPL values should be cited in units of dB re a reference value in micro Pa. When comparing sound pressure level values in air and in water with respect to loudness perception a reasonable approach would be to use sensation levels by subtracting the specimen's hearing threshold at a given frequency.

Sound exposure level (SEL): Sound exposure level is a measure of the potential energy of a sound and therefore depends on both amplitude and duration of a signal. It is the time integral of the instantaneous squared sound pressure normalized to a 1-s period. Sound exposure level can also be calculated from

SEL=sound pressure level +10 log (duration)

Temporary and permanent hearing damage are a function of exposure time and sound pressure level. Therefore, SELs are considered to be useful predictors for physiological impact of noise. Hearing damage is roughly proportional to the overall acoustic energy of a stimulus (equal energy hypothesis).

Sound pressure (SP):

Pressure (force [N] /area [m^2]) difference between maxima and minima of an acoustic wave expressed in Pascal

Temporary threshold shift: A temporary but fully recoverable upwards shift of the auditory threshold (a temporary loss of hearing sensitivity or “temporary partial deafness”). TTS can be caused by exposure to sounds for extended amount of times or short expsosure to high intensity stimuli. TTP is a function of both exposure time and sound pressure level (SPL).

Tone: The term refers to a sound that only contains energy at one frequency and has a sine-shaped waveform.

Waveform: Measured sound pressure fluctuation plotted against time. The waveform of a pure tone follows a sine shape.