Effects of naval sonar on seals

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English summary

There is a growing concern that active military sonars might injure or harm marine mammals. We have therefore done controlled exposure experiments on captive hooded seals to investigate behavioral, physiological and possible neuropathological effects.

The animals were instrumented with dataloggers recording heart rate, diving and swimming activity, before being released into a $1200m^3$ net cage in the ocean. The sonar exposure involved "soft start" and "slow start" procedures using simulated sonar signals between 1-7 kHz at source levels from 134-194dB (RMS, re1uPa@1m). The sound pressure level inside the net cage was 10-27dB below the source level.

After completion of the experiments the animals were autopsied and the brains examined for neuropathological effects of the exposure. We found that the animals initially responded with avoidance to signals above 160-170dB (received levels). This involved reduced diving activity, commencement of rapid (exploratory) shallow swimming and eventually displacement to areas of least sound pressure level. However, already upon the second exposure the initial rapid swimming activity was absent, while the reduction in diving activity became even more pronounced. No differences were found in behavioural response to different transmitted frequencies. Increased heart rate at the surface indicates emotional activation during sonar exposure, but lack of effect of sonar exposure on heart rate during diving indicates that physiological responses to diving remain intact. We found no sign of traumatic brain injury upon *post mortem* examination.

Sammendrag

De senere år har det utviklet seg en økende bekymring for at lydpulser fra militære sonarer kan påføre marine pattedyr direkte skade eller endre deres atferd på en måte som indirekte skader dem. Forskningsfokuset har vært på hval, men det er ingen åpenbar fysiologisk grunn til at ikke sel skulle bli like påvirket. Vi har derfor gjennomført kontrollerte eksponeringer av sel i fangenskap for å undersøke eventuelle atferdsresponser, fysiologiske responser og neuropatologiske effekter av militære sonarer.

Dyrene ble instrumentert med dataloggere som registrerte hjertefrekvens, dykk- og svømmeaktivitet før de ble sluppet ut i en 1200m^3 laksemerd i sjøen utenfor Olavsvern Orlogsstasjon i Tromsø. Selve eksponeringen besto i en "soft start" og en "slow start" prosedyre hvor man brukte simulerte sonarsignaler fra 1 til 7kHz med kildenivå fra 134 til 194dB (RMS, re1uPa@1m). Lydnivået i merden ble målt til 10-27dB under kildenivå.

Etter avsluttet eksperiment ble dyrene obdusert og hjernen ble undersøkt for å se etter tegn på neuropatologisk skade som en følge av eksponeringen. Våre resultater viser at når lydnivåene oversteg 160-170 dB under første eksponering reagerte dyrene med en orienteringsrespons som besto i hurtig svømming på eller nær overflaten, redusert dykkaktivitet og forflytning til områder med lavest lydnivå lengst bort fra sonarkilden. Allerede fra andre eksponering er denne orienteringsresponsen borte, men dyrene reagerer fortsatt med redusert dykkaktivitet og unnvikelse. Vi fant ingen forskjell i atferdsrespons mellom ulike frekvensområder innenfor det testede frekvensbåndet. Økt hjertefrekvens når dyrene eksponeres og ikke dykker, tyder på emosjonell aktivering (stress), men når dyrene dykker under eksponeringen reduseres hjertefrekvensen til normalt nivå under dykk, noen som tyder på at den fysiologiske dykkresponsen ikke påvirkes av sonaren. Vi fant ingen tegn til traumatisk skade i noen del av hjernen, verken knusingsskader, andre type blødninger, aksonal skade, infarkter, intravaskulær bobledannelse eller betennelser.

Contents

Preface

This study was conducted at the Olavsvern Naval Base outside Tromsø in 2005. The original objective was to study behavioural, physiological and pathological effects of naval sonar signals on seals. Four seals were brought from a wild animal research facility at the University of Tromsø to a large net pen in the ocean. In order to conduct a realistic experiment the animals were exposed to increasing levels of simulated sonar sounds up to levels which would correspond to levels less than 100 m distance from a naval sonar source.

After termination of the experiments the animals were put to death and the brain were examined for potential neurological injury. The necessity to terminate the animals was partly driven by the scientific hypothesis that sonar signals could inflict neurological injury, but was also required as part of the permit from the Norwegian Animal Research Authority (permit no 2004/11380).

A manuscript describing the results was submitted to a high impact scientific peer-review journal in 2006. However, despite the fact that the same journal publishes numerous articles which involves termination of other research animals, the editor and referees did not except inclusion of such data from a marine mammal. We eventually accepted that in some cultures marine mammals are, in the words of George Orwell, `more equal´ than other mammals. We therefore removed the autopsy data and submitted the results of the analysis of behavioural and physiological effects to a different journal. This article was published in the September 2010 issue of *Aquatic Mammals*, and it is re-printed as an appendix to this report with permission of the editor. However, this report includes also the results of the pathological examination.

1   Introduction

International scientific (IWC, 2004; SCAR, 2004; ICES, 2005) governmental (EU, 2004; IUCN 2004) as well as non-governmental (WDCS, 2003; NRDC, 2005) organisations have expressed concern that intense anthropogenic acoustic signals might harm marine mammals. A primary reason for this concern is several incidents of mass stranding of cetaceans, coinciding with the use of active sonar (Frantzis, 1998; D'Amico and Verboom, 1998; Balcomb and Claridge, 2001; Evans and England, 2001; Jepson *et al.,* 2003; Fernández *et al.,* 2005). Necropsy of stranded animals supposedly indicate acoustically induced trauma, including brain hemorrhages (Evans and England, 2001; Fernández *et al.,* 2005).

Acoustic resonance in air spaces connected to the airways, has been offered as explanation for these injures (Balcomb and Claridge, 2001). Whole-lung resonance frequency has been determined in humans (Martin *et al.,* 2000), bottlenose dolphins (*Tursiops truncatus*) and white whales (*Delphinapterus leucas*) (Finneran, 2003), and found to be below 100 Hz, even at depth. Signals in the mid-frequency range (1-7 kHz) would therefore have to be exceedingly powerful in order to cause any direct damage (Cudahy and Ellison, 2001). Even if injuries caused by resonance in air spaces at the moment can not be excluded, a workshop on the subject has concluded that it is not very likely (Evans, 2002). Cranial resonance has also been mentioned as a possible mechanism for inducing brain damage (Cudahy and Ellison, 2001). The resonance frequency of the human skull has been determined to 600-900 Hz underwater (Hanson and Cudahy, 1998), and since many seal and whale skulls are likely to have similar underwater resonance frequencies, brain damage from skull resonance is, at the moment, an intriguing possibility. Finally, it has been suggested that the stranded animals have suffered from some sort of decompression sickness as a result of exposure to sonar signals (e.g. Jepson *et al.,* 2003; Fernández *et al.,* 2005), either as a result of changes in their diving behavior, or as a direct effect of sonar signals on pre-existing bubble precursors in nitrogen-supersaturated tissue (Jepson *et al.,* 2003; 2005).

Most of the animals stranded in connection with the use of active military sonar have been beaked whales. The focus so far, has therefore been on cetaceans. However, no plausible explanation for why beaked whales should be more vulnerable than other marine mammals has been put forward. Pinnipeds have hearing abilities which equal, or even surpass, those of many cetaceans in this frequency range (1-7 kHz) (fig 1.1) (Møhl, 1968; Therhune and Ronald, 1972; 1975; Therhune, 1988; Kastak and Schusterman, 1998; Kastelein et al., 2009a&b). Moreover, seals are generally smaller than (most) cetaceans, which implies that their resonance frequencies are higher and correspond more closely to the transmitting frequency of mid-frequency (1-7 kHz) sonars than those of the larger whales, making seals, at least, as susceptible to sonar signals as cetaceans.
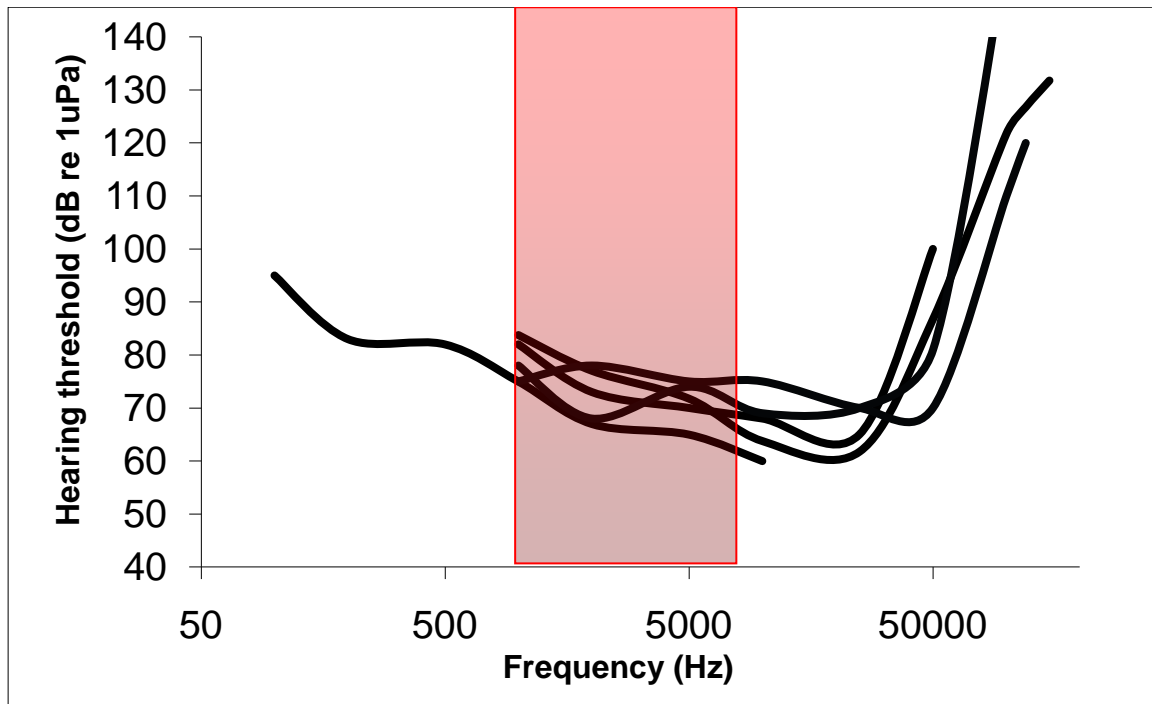
Figure 1.1   Hearing curves of phocid seals (harbor seals, harp seals and ringed seals) show that they have sensitive hearing in the typical frequency band of military sonars (1-7 kHz) (red shaded area). The source of the curves are referenced in the text.

Several existing regulations for deployment of high intensity acoustic sources have introduced a mitigation procedure generally termed "soft start" (military terminology) or "ramp-up" (civilian terminology) (JNCC, 1998; NATO URC, 2004; NOAA, 2002). This procedure implies that the sound pressure level is slowly increased to provide the marine mammals with an opportunity to evacuate the area before the source is operated at full power. If, however, marine mammals do not move away from the source, and instead ignore it or even approach it, the procedure may be counterproductive. We therefore suggest an alternative procedure termed "slow start", by which transmission starts at a high pressure level, using short pulses and long pulse intervals, allowing the animals to avoid the sound source before signal duration is increased and pulse interval decreased to what could be detrimental levels. This procedure is based on the assumption that for sonar signals it is the accumulated sound exposure level, rather than the peak pressure level, which lead to physiological effects. This assumption has been found to be true for the development of temporary hearing threshold shifts (tts) in marine mammals at signal durations relevant to tactical military sonars (Finneran et al 2002; 2003, 2005 and Schlundt et al 2000).

In the present study we describe the behavioural responses in hooded seals to both "soft" and "slow" start sonar exposure under controlled conditions in a free acoustic field in the ocean. During these experiments we also recorded the heart rate of the animals which were thereafter sacrificed and their brains examined for neuropathological effects.

2 Materials and Methods

2.1 Animals, instrumentation and data analysis

Details of animals, instrumentation and data analysis are given in Kvadsheim *et al.* 2010 (appendix A).

2.2 Experimental protocol

In preparation for the experiments, the instrumented animals were transferred to a floating 1,200 m^3 (diameter = 20 m; max depth = 8 m) net-cage (salmon fish farm) located in a fjord outside Tromsø (fig. 2.1). The net-cage had an internal wooden raft which could be accessed by the animal from all angles. The animals were used to being in groups, and therefore two animals were always together in the floating net-cage. The usual feeding routine was maintained throughout the study period.

After instrumentation, the animals were allowed a period of 4 and 7 days for animal pair 1 and 2, respectively, to acclimate to their new oceanic environment, after which they seemed well adapted and were eating normally. On the day of sonar signal exposure, surface activity was video-monitored using a camera which was placed above the net-cage and which could capture the entire cage continuously in one frame. These recordings were later used in the analysis of surface activity in relation to the position of the sonar source.

Figure 2.1   Experimental setup and animal instrumentation

First, a "soft start" procedure (Appendix A; Figure 2), consisting of a series of 1 s sonar pulses every 10 s (duty cycle 10%), gradually increasing in pressure level from 134 dB$_{RMS}$ to 194 dB$_{RMS}$ source levels (re 1uPa @ 1 m) in 10 dB steps within 6 min, was executed. The sound pressure level inside the net-cage was 10 to 27 dB below the source level (Appendix A; Figure 2). This procedure was repeated three times, each with a different linear frequency modulated up-sweep (1.3-1.7 kHz, 3.7-4.3 kHz and 6.0-7.0 kHz), with 10 min of silence between the different exposures. The three sweeps were presented in a random order to distinguish frequency-specific responses from a general adaptation to sonar exposure. Second, after one hour of silence, a "slow

start" procedure (Appendix A; Figure 2), consisting of a series of 1 s signals at 194 dB source level with increasing duty cycles from 1% (100 s signal interval) to 10% (10 s signal interval) in 10 min, was executed. This procedure was also repeated three times using the same series of frequency-modulated sweep signals in a random order. The choice of frequency-modulated up-sweep signals was made to closely mimic the most frequently used military sonar signals. The entire experiment was completed within 6 hours, on two different occasions with the two animal groups. After this protocol the seals were captured, immobilized (*i.m.* injection of Zoletil Forte Vet. 1.0 mg/kg) and killed (*i.v.* injection of Pentobarbital 15mg/kg). Their brains and brainstems were then carefully excised and fixed (section 2.3).

2.3   Neuropathology

Two of the animals were killed 20 hours, and the other two 3 days after exposure to allow for histopathological detection of possible tissue damage. In addition to the 4 exposed animals, two unexposed animals of the same species and age were killed for comparison between exposed and un-exposed individuals. Immediately after death the brains, including the brainstem and upper part of the cervical spinal cord, were carefully excised and immersed in 4% buffered formalin for 5 weeks. The surface of the brains was then carefully inspected for hemorrhages and other traumatic lesions. The cerebral hemispheres, brainstem and cerebellum were separated. The right cerebral hemisphere was preserved for possible futures studies. The left cerebral hemisphere and *cerebellum* were cut in 5 mm thick coronary sections and the brainstem in 3 mm transversal sections. All slices were closely inspected for structural abnormalities. Six of the coronary sections from the left cerebral hemisphere were routinely processed and embedded in paraffin. These sections allowed for microscopic examination of cortex and white matter from all lobes as well as periventricular white matter, *corpus callosum, thalamus*, *hypothalamus,* basal ganglias and *hippocampus.* Sections from the left *bulbus olfactorius, vermis cerebelli,* left *nucleus dentatus* as well as every second section from the brainstem were also paraffin-embedded. Five μm thick sections were cut, stained with hematoxylin-eosin (H&E) and luxol fast blue and examined by a senior neuropathologist using light microscopy.

3   Results

3.1   Behavioral and physiological effects of sonar exposure

Results of analysis on behavioral and physiological effects of sonar exposures are given in Kvadsheim *et al.* 2010 (Appendix A).

3.2   Neuropathological effects of sonar exposure

Macroscopic examination: Immediate inspection upon removal of the scull did not reveal extra- or subdural hematomas in any of the cases. There were neither leptomeningeal hemorrhages, contusions nor signs of herniation in any of the brains. All six brains demonstrated symmetric

cerebral hemispheres. A slightly compressed ventricular system was observed in two of the exposed animals. The cut sections did not reveal any additional findings.

Microscopic examination: Hemorrhages or axonal swellings were not found in any of the sections. Moreover, infarcts, selective neuronal necrosis, intravascular gas bubbles or signs of inflammation were not observed. Areas of polymicrogyria as well as neuronal heterotopias in the leptomeninges and cerebral white matter were observed in all six brains.

4   Discussion

4.1   Behavioral effect of sonar

This study showed that young hooded seals started to show active avoidance behaviour in response to 1-7 kHz sonar signals transmitted at 10% duty cycle at received sound pressure levels above 160-170 dB$_{RMS}$ (re 1µPa). The lack of response to sound below this level is worth noticing in view of the fact that this level is well above the hearing threshold (54-80 dB (re 1 µPa)) of seals in the frequency range of 1-7 kHz (Møhl, 1968; Terhune & Ronald, 1972, 1975; Terhune, 1988; Kastak & Schusterman, 1998; Kastelein *et al* 2009a&b). In this context it is also worth noticing that free-ranging elephant seals (*Mirounga angustirostris*) showed no change in diving behaviour when exposed to very low-frequency signals (55-95 Hz) at levels up to 137 dB (Costa et al., 2003), and that trained captive sea lions (*Zalophus californianus*) showed avoidance behaviour in response to impulse sounds at levels above 165-170 dB$_{RMS}$ (Finneran et al., 2003). The initial response of our animals was to increase swimming activity at the surface (Appendix A; Figure 5B), apparently to seek out areas of minimum sound pressure level (Appendix A; Figure 3). All animals rapidly adapted to the exposure: Already at the second exposure trial, the increase in swimming activity was no longer evident (Appendix A; Figure 5B), and the reduction in diving activity and floating with the head out of water became more conspicuous at every exposure (Appendix A; Figure 5A). Sound conduction pathways for underwater hearing in pinnipeds are not fully understood, but lifting the head out of the water, reduced diving activity and increased surface time may be a way to reduce exposure to unpleasant or painful sound levels, as well as the risk of hearing injury.

The frequency-modulated up-sweep signals used were chosen because of their operational relevance. Up-sweeps may have a Doppler perception for the animal, as if the sound source is rapidly approaching. It cannot be ruled out that the initial response is in part due to this phenomenon, and that a different sonar signal (e.g., a continuous wave or a down-sweep signal) would result in a different response even with the same frequency band. The sonar source used did contain some upper harmonics, when transmitting at the maximum source level, particularly at the lower frequency sweep (1-2 kHz). However, even the second upper harmonic of the lowest fundamental was attenuated by at least 30 dB, and for the highest fundamental frequencies the second harmonic was attenuated by at least 50 dB. Since the hearing curve of phocid seals is flat within the band from 200 Hz to at least 40 kHz (e.g. *Kastelein et al* 2009b, Fig 1.1), this would

imply that the loudness of the fundamentals would completely dominate over the harmonics. It is therefore highly likely that it was the fundamental signals which triggered responses, not harmonics. In fact, our result shows that within the tested band there is no frequency dependency of the response (Appendix A; Figure 4), which again is not surprising given the flat hearing curve of these animals within this band (e.g. Kastelein *et al* 2009b, Fig 1.1),

In this study we did not have enough animals at our disposal to be able to rotate the "slow"- and "soft start" exposure protocols on naive animals, and thereby properly evaluate if one procedure is significantly different from the other in eliciting avoidance behaviour (Appendix A; Figure 5). It is to be expected, however, that avoidance reactions will be elicited at longer distances in the wild if "slow start", instead of "soft start" is applied, since the threshold of avoidance is then reached at a longer distance from the source.

4.2 Physiological effects of sonar

In the baseline control period, heart rate varied in a normal pattern with diving activity (Appendix A; Figure 2 and 6), while the average (diving and non-diving) heart rate increased by 34% during sonar exposure periods compared to the baseline period. Since the animals spent significantly less time diving during the exposures, most of this increase in heart rate is probably caused by this change in diving behaviour. However, even though the effect of diving is much stronger, a significant effect on heart rate was also found for sonar exposure as well as for the interaction between diving and sonar (Appendix A; Table 3). Heart rate is acknowledged as an indicator of the emotional status of an animal (e.g. Blix et al. 1974), but heart rate also increases with physical (swimming) activity, and in habitually diving animals it is often dramatically reduced during diving (e.g. Ramirez et al., 2007). It is therefore to be expected, as indeed observed in this study, that when the animals spent more time at the surface in response to sonar exposure, this resulted in increased heart rates (Appendix A; Figure 6). However, our results also show that when the animals were at the surface, the heart rate was increased during sonar exposure compared to the control period (Appendix A; Table 3). After the initial exploratory response, the activity level during exposure was comparable to or lower than the activity level during the control period (Appendix A; Figure 5), and thus there was no increase in physical activity which could explain the increased heart rate during exposure. The increased heart rate at the surface during sonar exposure might therefore indicate emotional activation or discomfort. However, the initial exploratory response followed by rapid behavioural adaptation with passive floating at the surface during sonar exposure, indicates that there was no panic. The lack of effect of sonar exposure on heart rate during diving (Appendix A; Table 3, Figure 6) also indicates that despite any emotional activation, normal physiological responses to diving were still intact.

It has been suggested that marine mammal stranding events that have coincided with the use of active military sonars may be explained by decompression sickness in that the signals trigger a change in the behavior of the animals, which in turn increases the risk of developing decompression sickness (Jepson *et al.,* 2003). The risk of such calamities would be particularly prominent in animals which already have supersaturated tissues due to extensive diving (Ridgway and Howard, 1979). If such animals suddenly change behavior and start spending unusually much

time close to the surface, where the nitrogen absorption and saturation level will be highest, this might increase the risk to the animal. Moreover, if such behavior is combined with vigorous swimming to avoid the acoustic signals, the risk would further increase (Pollard *et al.,* 1995). The behavioral responses of the seals examined in the present study included both cessation of diving, increased time spent at the surface and an initial increase in swimming activity. Even so, no gas bubbles were found during the pathological examination of the seal brains in the present study, but the risk to free-ranging animals that may perform deeper dives, still needs to be explored further.

4.3   Pathological effects of sonar

Acoustic resonance in air spaces (Balcomb and Claridge, 2001) or bone structures (Cudahy and Ellison, 2001) has been proposed as yet another causal link between sonar exposure and tissue damage in stranded cetaceans (Evans and England, 2001). The size of pinnipeds might make them more vulnerable to injury caused by acoustic resonance, because their resonating structures probably have resonance frequencies closer to the transmitting frequency of the sonars. Still, we found no sign of traumatic brain injury in our seals, which were repeatedly exposed to levels above 180 dB. This is in support of a workshop on the subject, that concluded that it is unlikely that acoustic resonance caused the stranding events (Evans, 2002).

An interesting finding in our neurological examination is areas of polymicrogyria as well as neuronal heterotopias in the leptomeninges and cerebral white matter of the seal brain. Such features are considered to be congenital deformities in humans and are often associated with epilepsy. However, this was observed in all six seals brains, including the unexposed control brains, and therefore has to be considered normal. To our knowledge this has not been described in seals before. The potential functional relevance of these abnormalities, if any, remains unknown.

It is assumed that the hearing organ is very sensitive towards acoustic energy. It has been demonstrated in odontocetes that it is the total sound exposure level that determines the threshold of temporary hearing threshold shifts (TTS) (Finneran *et al.,* 2002), and this seems to apply also to pinnipeds (Finneran *et al.,* 2003). This implies that a short duration signal will have to have a higher pressure level than a long duration signal in order to inflict the same TTS. Accordingly, Schlundt *et al.* (2000) found that the signal level necessary to induce TTS in odontocetes were 192-201 dB with signals of the same duration (1 s) and frequencies (3-20 kHz) as in the present study. TTS in pinnipeds have only been determined in animals exposed to very long duration octave-band noise (Kastak *et al.,* 1999). If, for the sake of the argument, one extrapolates from this, and employs the "equal-energy criteria" established by Finneran *et al.* (2002; 2003), a 1 s signal would require a sound pressure of at least 190 dB in order to induce TTS in pinnipeds. In the present study, pressure levels of this magnitude were only present in the immediate surroundings of the transducer, and bearing in mind that a TTS, by definition, is fully reversible, it is highly unlikely that the animals employed in the present study suffered any TTS, let alone a permanent hearing injury.

5   Conclusions

Mid-frequency sonar signals (1-7 kHz) transmitted at 10% duty cycle elicited active avoidance behaviour in hooded seals at received sound pressure levels exceeding 160-170 dB$_{RMS}$ (re 1μPa). The behavioural response involved reduced diving and initial swimming away from the sonar source, followed by rapid behavioural adaptation resulting in passive floating at the surface. No differences were found in behavioural responses in relation to transmitted frequency within the 1-7 kHz range tested. Increased heart rate at the surface, which is not explained by increased swimming activity, indicates emotional activation during sonar exposure, but lack of effect of sonar exposure on heart rate during diving indicates that physiological responses to diving remain intact. Direct pathological effects of exposure, including hearing loss, are unlikely to occur at levels below 190 dB.

We suggest that the "slow start", proposed and tested here, is more favourable than the "soft start / ramp up" procedure, since it will make the animal aware of the sound at a longer distance from the source.

6   References

Balcomb, K. C., and Claridge, D. E. (**2001**). "A mass stranding of cetaceans caused by naval sonar in the Bahamas". Bahamas J. Sci. **5**, 2-12.

Blix, A. S., Strømme, S. B., and Ursin, H. (**1974**). "Additional heart rate – An indicator of psychological activation". Aerospace Med. **45**, 1219-1222.

Costa, D., Crocker, D. E., Gedamke, J., Webb, P. M., Houser, D. S., Blackwell, S. B., Waples, D., Hayes, S. A., and Le Boeuf, B. J. (**2003**). "The effect of a low-frequency sound source (acoustic thermometry of the ocean climate) on the diving behavior of juvenile northern elephant seals, *Mirounga angustirostris*". J. Acoust. Soc. Am. **113,** 1155-1165.

Cudahy, E., and Ellison, W. T. (**2001**). "A review of the potential for *in vivo* tissue damage by exposure to underwater sound". In: *Environment impact statement for surveillance towed array sensor system low frequency active (SURTASS LFA) sonar.* Report from Department of the Navy, Chief of Naval Operations, USA.

D'Amico, A., and Verboom, V. C. (**1998**)**.** "Report of the bioacoustics panel convened at SACLANTCEN 15-17 July 1998". SACLANTCEN **M-133.**

EU (**2004**). "European Parliament resolution on the environmental effects of high-intensity active naval sonars". **B6-0089/04.**

Evans, D. L., and England, G. R. (**2001**). "Joint interim report – Bahamas marine mammal stranding event of 15-16 March 2000". Report from Dept. of Commerce, NOAA and Secretary of the Navy, USA.

Evans, D. L. (**2002**). "Report of the workshop on acoustic resonance as a source of tissue trauma in cetaceans. April 24 and 25, 2002, Silver Spring, MD, USA". Report from Dept. of Commerce, NOAA.

Fernandez, A., Edwards, J. F., Rodriguez, F., Espinosa de los Monteros, A., Herraez, P., Castro, P., Jaber, J. R., Martin, V. and Abbelo, M. (**2005**). "Gas and fat embolic syndrome involving a

mass stranding of beaked whales (family *Ziphiidae*) exposed to anthropogenic sonar signals". Vet. Pathol. **42,** 446-457.

Finneran, J. J., Dear, R., Carder, D. A. and Ridgway, S. A. (**2003**). "Auditory and behavioral responses of California sea lions to single underwater impulses from an arc-gap transducer". J. Acoust. Soc. Am. **114,** 1667-1677.

Finneran, J. J., Schlundt, C. E., Dear, R., Carder, D. A. and Ridgway, S. H. (**2002**). "Temporary shift in the masked hearing thresholds in odontocetes after exposure to single underwater impulses from a seismic watergun". J. Acoust. Soc. Am. **111,** 2929-2940.

Finneran, J. J., Carder, D. A., Schlundt, C. E. and Ridgway, S. H. (**2005**). Temporary threshold shift in bottlenose dolphins (*Tursiops truncatus)* exposed to mid-frequency tones. J. Acoust. Soc. Am. **118,** 2696-2704.

Frantzis, A. (**1998**). "Does acoustic testing strand whales"? Nature **392,** 29.

Hanson, E. and Cudahy, E. (**1998**). "Skull vibration in the presence of waterborne low frequency sound". In: *Proceedings of noise effects '98, The 7th International Congress on noise as a public health problem.* **1,** 298-301.

ICES (International Council for the Exploration of the Sea) (**2005**). "Report of the ad-hoc group on the impact of sonar on cetaceans and fish". ICES CM 2005/ACE:01.

IUCN (International Union for Conservation of Nature) (**2004**). "Undersea noise pollution. Resolution" RES **3.068**.

IWC (International Whaling Commission) (**2004**). "Report of the scientific committe 2004".

Jepson, P. D., Arbelo, M., Deaville, R., Patterson, I. A. P., Castro, P., Baker, J. R., Degollada, E., Ross, H. M., Herráez, P., Pocknell, A. M., Rodriguez, F., Howie, F. E., Espinosa, A., Reid, R. J., Jaber, J. R., Martin, V., Cunningham, A. A. and Fernández, A. (**2003**). "Gas-bubble lesions in stranded cetaceans". Nature **425,** 575-576.

JNCC (Joint Nature Conservation Committee) (**1998**). "Guidelines for minimising acoustic disturbances to marine mammals from seismic surveys". Joint Nature Conservation Committee, UK.

Kastak, D. and Schusterman, R. J. (**1998**). "Low-frequency amphibious hearing in pinnipeds: Methods, measurements, noise, and ecology". J. Acoust. Soc. Am. 103, 2216-2228.

Kastak, D., Schusterman, R. J., Southall, B. L. and Reichmuth, C. J. (**1999**). "Underwater temporary threshold shift induced by octave-band noise in three species of pinniped". J. Acoust. Soc. Am. 106, 1142-1148.

Kastelein, R. A., Wensveen, P. J., Hoek, L., Verboom, W. C., and Terhune J. M. (**2009a**). Underwater detection of tonal signals between 0.125 and 100 kHz by harbor seals (Phoca vitulina), J. Acoust. Soc. Am. 125, 1222-1229.

Kastelein, R. A., Wensveen, P., Hoek, L., Terhune, J. M. (**2009b**). Underwater hearing sensitivity of harbor seals (Phoca vitulina) for narrow noise bands between 0.2 and 80 kHz, J. Acoust. Soc. Am. 126, 476-483.

Kvadsheim, P.H., Sevaldsen, E.M., Folkow, L.P. and Blix, A.S (**2010**). Behavioral and physiological responses of hooded seals (*Cythophora cristata*) to 1 to 7 kHz sonar signals. Aquatic Mammals 36(3), 239-247.

Martin, J. S., Roger, P. H., Cudahy, E and Hanson E. (**2000**). "Low frequency responses of the submerged human lung". J. Acoust. Soc. Am. **107**, 2813.

Møhl, B. (**1968**). "Auditory sensitivity of the common seal in air and water". J. Auditory Res. **8,** 27-38.

NATO URC (North Atlantic Treaty Organization Underwater Research Center) (**2004**). "NATO URC Human Diver and marine mammal risk mitigation rules". NATO Underwater Research Center, staff instruction **77**.

NOAA (National Oceanic and Atmospheric Administration) (**2002**). "Taking and importing marine mammals; Taking marine mammals incidental to navy operations of surveillance towed array sensor system low frequency active sonar". NMFS (National Marine Fisheries Service), NOAA, Dept. Commerce, USA**. 50** CFR Part **216.**

NRDC (National Resources Defense Council) (**2005**). "Sounding the depth II: The rising toll of sonar, shipping and industrial ocean noise on marine life". Jasny, M., Reynolds, J., Horowitz, C. and Wetzler, A. (eds).

Pollard, G. W., Marsh, P. L., Fife, C. E., Smith, L. R. and Vann, R. D. (**1995**). "Ascent rate, post-dive exercise, and decompression sickness in the rat". Undersea Hyper. Med. **22,** 367-376.

Ramirez, J.-M., Folkow, L. P. and Blix, A. S. (**2007**). "Hypoxia tolerance in mammals and birds: From the wilderness to the clinic". Annu. Rev. Physiol. **69**, 113-143.

Ridgway, S. H. and Howard, R. (**1979**). "Dolphin lung collapse and intramuscular circulation during free diving: Evidence from Nitrogen washout". Science **206**, 1182-1183.

SCAR (Scientific Committee on Antarctic Research) (**2004**). "SCAR report on marine acoustic technology and the Antarctic environment". XXVII ATCM (Antarctic Treaty Consultative Meeting), May 2004, Cape Town, South Africa. Information Paper **IP 078.**

Schlundt, C. E., Finneran, J. J., Carder, D. A. and Ridgway, S. H. (**2000**). "Temporary shift in masked hearing thresholds of bottlenose dolphins and white whales after exposure to intense tones". J. Acoust. Soc. Am. **107,** 3496-3508.

Terhune, J. M. (**1988**). "Detection thresholds of a harbour seal to repeated underwater high-frequency, short-duration sinusoidal pulses". Can. J. Zool. **66,** 1578-1582.

Terhune, J. M. and Ronald, K. (**1972**). "The harp seals, *Phagophilus groenlandicus (*Erxleben, 1777). III The underwater audiogram". Can. J. Zool. **50,** 565-569.

Terhune, J. M. and Ronald, K. (**1975**). "Masked hearing thresholds of ringed seals". J. Acoust. Soc. Am. **58,** 515-516.

WDCS (Whale and Dolphin Conservation Society) (**2003**). "Oceans of noise - a WDCS science report". Simmonds, M., Dolman, S. and Weilgart, L (eds)**.**

Appendix A

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Kvadsheim, P.H., Sevaldsen, E.M., Folkow, L.P. and Blix, A.S (**2010**). Behavioral and physiological responses of hooded seals (*Cythophora cristata*) to 1 to 7 kHz sonar signals. Aquatic Mammals 36(3), 239-247, DOI 10.1578/AM.36.3.2010.239.

Behavioural and Physiological Responses of Hooded Seals (*Cystophora cristata*) to 1 to 7 kHz Sonar Signals

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Abstract

Controlled exposure experiments on captive hooded seals (*Cystophora cristata*) were made to examine behavioural and physiological effects of sonar signals. The animals were instrumented with data loggers recording heart rate, dive depth, and swimming activity, and then released into a 1,200 m³ net-cage in the ocean. The exposure consisted of three different 1-s sonar signals covering the 1 to 7 kHz band transmitted either by using 10-s inter-ping intervals and gradually increasing source level from 134 to 194 dBRMS (re 1 μPa @ 1 m) within 6 min, or using the maximum source level of 194 dBRMS from the first ping but gradually decreasing the inter-ping intervals from 100 s to 10 s within 10 min (duty cycle increasing from 1 to 10%). Transmission loss from the source to the animal varied from 10 to 27 dB, depending on the exact location within the net-cage and the transmitted frequency. The animals responded to the initial (10% duty cycle) exposure with avoidance to signals above 160 to 170 dBRMS (re 1 μPa) received levels. This involved reduced diving activity, commencement of rapid exploratory swimming at surface, and eventually displacement to areas of least sound pressure level. However, already upon the second exposure, the initial rapid swimming activity was absent, while the reduction in diving activity became even more pronounced. No differences were found in behavioural response to different transmitted frequencies. Increased heart rate at the surface indicates emotional activation during sonar exposure, but lack of effect of sonar exposure on heart rate during diving indicates that physiological responses to diving remain intact.

Key Words: active sonar, marine mammals, behaviour, heart rate

Introduction

International scientific (International Whaling Commission [IWC], 2004; Scientific Committee on Antarctic Research [SCAR], 2004; International Council for the Exploration of the Sea [ICES], 2005), governmental (European Union [EU], 2004; International Union for the Conservation of Nature [IUCN], 2004), as well as nongovernmental (Simmonds et al., 2003; Jasney et al., 2005) organisations have expressed concern that intense anthropogenic acoustic signals might harm marine mammals. A primary reason for this concern is several incidents of mass stranding of cetaceans coinciding with the use of active sonar (D'Amico & Verboom, 1998; Frantzis, 1998; Balcomb & Claridge, 2001; Evans & England, 2001; Jepson et al., 2003; Fernández et al., 2005).

In this report, we are not dealing with the direct causes of mass strandings of cetaceans but are instead investigating the behavioural and physiological responses of hooded seals (*Cystophora cristata*) to direct exposure to military sonar signals in the 1 to 7 kHz band in order to assess potential adverse effects. Pinnipeds have hearing abilities which equal, or even surpass, those of many cetaceans in this frequency range (Møhl, 1968; Terhune & Ronald, 1972, 1975; Terhune, 1988; Kastak & Schusterman, 1998; Kastelein et al., 2009a, 2009b) and are, based on hearing sensitivity, potentially at least as sensitive to sonar signals as cetaceans.

Materials and Methods

Animals and Upkeep
The experiments involved four 1-y-old hooded seals caught as pups in the pack ice off East-Greenland and raised in captivity in 45,000-l seawater pools at the University of Tromsø. The animals (two males and two females, weighing 64 to 84 kg) were offered herring (*Clupea harrengus*) supplemented with a vitamin complex once every day. The animals were collected under

permits issued by The Royal Norwegian Ministry of Fisheries, and the experiments were carried out under permit from the Norwegian Animal Research Authority (Permit No. 2004/11380) in compliance with ethical use of animals in experimentation.

Instrumentation
Prior to the experiments, the animals were instrumented under sedation (i.m. injection of 1.0 mg·kg^{-1} Zoletil Forte Vet. (tiletamin-zolazepam, Virbac, Carros Cedex, France) with dataloggers capable of recording diving behaviour, swimming activity, and heart rate. Two subcutaneous electrodes connected to insulated copper leads were surgically implanted 15 cm apart along the dorsal midline just posterior to scapulae under additional local anaesthesia (s.c. injection of 2 to 3 ml 10 mg·ml^{-1} Xylocain (AstraZeneca, Södertälje, Sweden). The leads were connected to a heart rate transmitter placed on top of a heart rate receiver and logger (HRX/HTR, Wildlife Computers, Redmond, WA, USA). The HRX/HTR unit and a time depth recorder (MK9, Wildlife Computers) were subsequently put into a specially designed mount (50·80·32 mm; 400 g), which was glued to the fur behind the scapulae using fast-setting epoxy resin. The loggers were set to record heart rate and dive depth every second. In addition, the gross motor (swimming) activity of the animals was recorded continuously with activity loggers (Actiwatch, MiniMitter, Bend, OR, USA) that were placed inside a waterproof cylindrical container (Ø = 63 mm, l = 20 mm, 70 g) that were glued to the fur in the dorsal midline over the pelvis. The activity loggers contained an omni-directionally sensitive accelerometer (sensitivity 0.05 g/0.49 m·s^{-2}), which measured motion-induced voltage changes at 32 Hz and converted these into values (counts) that were integrated over sampling periods (bins) of 30 s.

In preparation for the experiments, the instrumented animals were transferred to a floating 1,200 m^3 (diameter = 20 m; max depth = 8 m) net-cage (salmon fish farm) located in a fjord outside Tromsø. The net-cage had an internal wooden raft (Figure 1), which could be accessed by the animal from all angles. The animals were used to being in groups, and, therefore, two animals were always together in the floating net-cage. The usual feeding routine was maintained throughout the study period.

After instrumentation, the animals were allowed a period of 4 and 7 d for animal pair 1 and 2, respectively, to acclimate to their new oceanic environment after which they seemed well-adapted and were eating normally. On the day of sonar signal exposure, surface activity was video-monitored using a camera which was placed above the net-cage and which could capture the entire cage continuously in one frame. These recordings were later used in the analysis of surface activity in relation to the position of the sonar source.

Acoustics
The sonar transducer used to generate simulated sonar signals (ITC-2015, International Transducer Corporation, Santa Barbara, CA, USA) was placed outside the net-cage at 5 m depth, 2 m from the net wall. A waveform generator (Hewlett Packard 33120A, Palo Alto, CA, USA) was used to generate a trigger pulse at every transmission. This triggered a second waveform generator (Agilent Technologies 33250A, Palo Alto, CA, USA) to generate three different 1,000-ms linear frequency-modulated up-sweeps (1.3 to 1.7 kHz, 3.7 to 4.3 kHz, or 6.0 to 7.0 kHz), which were fed into a power amplifier (L-50, Instruments Inc, San Diego, CA, USA) connected to the transducer using fade-in/fade-out on zero sine. A calibrated hydrophone with amplifier (Type 8104 with Nexus 2692, Brüel & Kjær, Nærum, Denmark), placed 3 m from the source, was used to measure the transmitted source level. The measured levels at 3 m distance were converted to the standard reference distance of 1 m assuming spherical spreading (i.e., transmission loss from 1 to 3 m equals 20log3). The sonar signals were recorded using a 16-bit resolution AD-converter at a sampling rate of 16 kHz (Sound Blaster Audigy 2NX, Creative Technology Ltd) connected to a laptop computer installed with analysis software (*Cool Edit 2000*, Syntrillium Software Corp., Phoenix, AZ, USA). The measured signals are given as equivalent broadband (0 to 8 kHz) sound pressure levels over the duration of the signal. The recording system was calibrated by feeding a 1 Volt RMS sinus pulse from the waveform generator into the AD-converter. Prior to experiments, the sonar system was tested using a higher sampling rate (up to 48 kHz) in order to record the possible existence of upper harmonics, and the transmission loss from the sonar source through the net-cage was measured for all three signal frequencies in 16 positions inside the net (Figure 1). In addition, sound speed profiles through the water column were recorded using a STD/CTD (model SD204, SAIV AS, Bergen, Norway). The profiles and instrumentation details were used as input into an acoustic model (LYBIN) to visualize the sound field inside the net (Figure 1). The LYBIN model was developed by Svein Mjølsnes at the Norwegian Defense Logistics Organization in collaboration with coworkers at the Norwegian Defence Research Establishment (FFI).
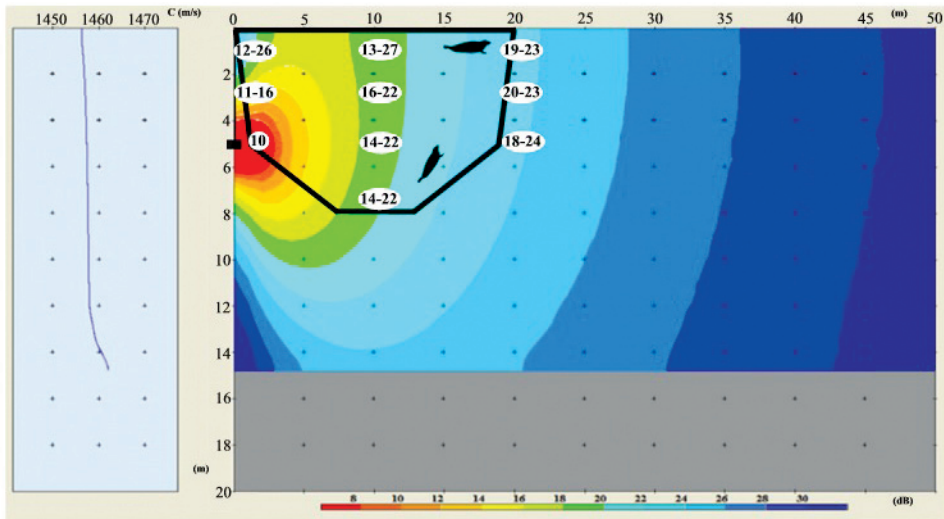
Figure 1. Sound picture inside the net-cage (right); the source was placed at 5 m depth (on the left side in the figure), and the coloration illustrates transmission loss along a vertical plane through the cage, based on an acoustic model (LYBIN) and the measured sound speed profile (left). The numbers signify measured transmission loss using a calibrated hydrophone. The measured intervals reflect variations among the different signal frequencies. In addition to the numbers shown, transmission loss was also measured at three positions in front of and behind the vertical plane shown.

Experimental Protocol

First, a "soft start" procedure (Figure 2), consisting of a series of 1-s sonar pulses every 10 s (duty cycle 10%), gradually increasing in pressure level from 134 to 194 dB$_{RMS}$ source levels (re 1 µPa @ 1 m) in 10 dB steps within 6 min, was executed. The sound pressure level inside the net-cage was 10 to 27 dB below the source level (Figure 1). This procedure was repeated three times, each with a different linear frequency modulated upsweep (1.3 to 1.7 kHz, 3.7 to 4.3 kHz, and 6.0 to 7.0 kHz), with 10 min of silence between the different exposures. The three sweeps were presented in a random order to distinguish frequency-specific responses from a general adaptation to sonar exposure. Second, after 1 h of silence, a "slow start" procedure (Figure 2), consisting of a series of 1-s signals at 194 dB source level with increasing duty cycles from 1% (100-s signal interval) to 10% (10-s signal interval) in 10 min, was executed. This procedure was also repeated three times using the same series of frequency-modulated sweep signals in a random order. The choice of frequency-modulated up-sweep signals was made to closely mimic the most frequently used military sonar signals. The entire experiment was completed within 6 h on two different occasions with the two animal groups.

Data Analysis

Based on data from the time-depth recorder (measured every 1 s), the diving frequency and the amount of time spent at the surface (depth ≤ 1 m), were calculated for the different experimental conditions. The data from the activity loggers are relative values of activity which are not only dependent on the specific level of activity of the animal but also on the exact position of the logger. To compare activity data among animals, a relative activity was therefore defined where the mean activity in the 1-h period just prior to exposure was defined as 100% for each animal. In addition, *surface events*, defined as an animal surfacing or staying at the surface for 30 s, were identified by use of continuous video recording before and during exposure. The net-cage was imagined to be divided into five zones, in addition to the floating raft (Figure 3), and the number of surface events in each zone was determined during the different experimental conditions.

Repeated measure ANOVA tests were used to analyse if the dependent variables describing behaviour and physiological responses varied with sonar exposure. Relative activity, the amount of time spent at the surface, and diving frequency were tested against sonar signal type (1.3 to 1.7 kHz, 3.7 to 4.3 kHz, 6.0 to 7.0 kHz, or no signal control) and exposure order (0 to 6, where 0 is no signal control). The experimental groups, which consisted of individual animals exposed together (two animals in each group), were used as between-factor grouping variables. For surface events, the different zones of the net-cage were used as the grouping variable to test if the number of surface events varied among zones and
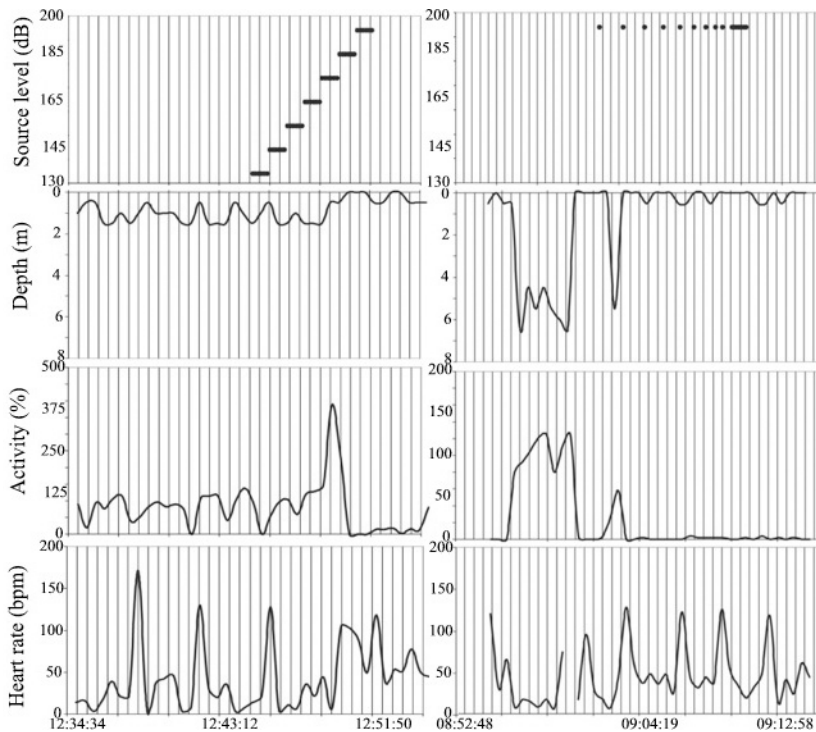
Figure 2. Typical raw data examples of dive depth (m), heart rate (bpm), swimming activity (arbitrary units), and transmitted source levels (dB$_{RMS}$ re 1 µPa @ 1 m) prior to and during exposure to sonar signals in a hooded seal; the left column is 10 min prior to, 10 min of sonar exposure using the "soft start" procedure, and 10 min after exposure, and the right column is 10 min prior to, 10 min of sonar exposure using the "slow start" procedure, and 10 min after exposure. Note pronounced bradycardia during diving and typical bimodal changes in heart rate caused by periods of spontaneous apnea when the animal is "resting" at the surface.

with the different experimental conditions. For the heart rate analysis, the diving state of the animal (submerged or not) was used as the grouping variable to enable the distinction between heart rate responses caused by the sonar from normal cardiac responses caused by diving. Data from only two of the animals were included in the heart rate analysis because the heart rate sensor malfunctioned in one animal and the depth sensor, which provided the diving state of the animal, malfunctioned in another animal. Fisher's Protected Least Significant Difference test was used as post hoc test. A $p < 0.05$ was considered to be significant.

Results

Behaviour
The four animals displayed two very different patterns of diving behaviour prior to exposure. Two of the animals, one in each of the two groups, spent less than 20% of their time at the surface (depth ≤ 1 m) and dived repeatedly to the bottom of the net-cage (8 m), while the other two spent more than 50% of their time at the surface.

During the initial gradual increase in transmitted source level, no obvious reaction was seen until source levels of 184 to 194 dB$_{RMS}$ (re 1 µPa @ 1 m) were reached. These source levels corresponded to received levels of 160 to 170 dB at the swimming locations of the animals. At these levels, all four animals displayed active avoidance behaviour which invariably involved reduction of diving activity followed by rapid swimming at the surface and eventually passive floating with the head out of water in areas with minimum sound pressure levels. Moreover, upon repeated exposure and regardless of signal frequency (Figure 4), all animals adapted to the exposure with disappearance of the initial exploratory swimming (Figure 5B) and direct transition from diving to passive floating at the surface (Figure 5) in the zone furthest from the sound source (Figure 3).

Repeated measure ANOVA tests show that the response to exposure, although always resulting in less time spent diving, did not involve any significant change in diving frequency (Table 1). The amount of time spent at the surface increased during exposure in all animals, and a significant

Table 1. Repeated measure ANOVA table; the within-group main effects of *exposure order* and *signal type* on the dependent variables *activity*, *time at surface*, and *dive frequency* were tested. The experimental groups are used as the between-factor grouping variable. Each experimental group consisted of individual animals being exposed together (two animals in each group). A significant within-group main effect of exposure order was found for activity, and a main effect of signal type was found for time at surface. Between-group main effects were never significant, indicating that the experimental groups behaved similarly. * signifies significant variance. Interaction bar plots are shown in Figures 4 and 5.

| | Dependent variables | | | | | |
| | Activity | | Time at surface | | Dive frequency | |
Factors	F-value	P-value	F-value	P-value	F-value	P-value
Exposure order	4.9	0.04*	2.1	0.20	1.5	0.32
Signal type	1.9	0.24	10.1	0.04*	1.6	0.36

main effect of signal frequency was found (Table 1) for this variable. However, it is evident from the interaction bar plot (Figure 4) that the significant effect of signal frequency is caused by the difference between baseline control (no signal) and exposure, independent of signal frequency, and not by any frequency-specificity in the response. A significant main effect of exposure order on the swimming activity was also found (Table 1). The interaction bar plot (Figure 5B) confirms our

Figure 3. The net-cage is imagined to be divided into five zones, as indicated by the dotted lines, in addition to the central wooden raft (rectangle). The horizontal position of the sonar source relative to the different zones is also indicated. The bars show the number of surface events within each zone in 10-min periods prior to sonar exposure (white bars) and during sonar exposure (black bars). A bar corresponding to three surface events within a 10-min period is placed outside the net-cage as a reference. Error bars signify the 95% confidence interval. Results of ANOVA tests are summarized in Table 2.

observation that the first exposure (independent of the frequency used) triggered an exploratory response with increased swimming activity, but the animals rapidly adapted to the sound, and this exploratory response was not seen during subsequent exposures. There was also a clear tendency of an order effect for surface activity (Figure 5A), which increased with the number of exposures, although this effect was not significant (Table 1). Our experimental design, where "soft start" was always executed before "slow start," does not allow us to distinguish the effects of the experimental condition ("soft start" vs "slow start") from order effects. However, the order effect is clearly evident from the interaction bar plot (Figure 5) already during the "soft start" exposures.

For surface events, the data were grouped according to the different zones of the net-cage (Figure 3), and numbers of surface events in the different zones were analysed with or without the sonar. Significant effects of both sonar and zone, as well as for the interaction between zone and sonar, were found (Table 2). This implies that there was an increase in the number of surface events during the exposure periods and a zone preference element in the behaviour of the animals (Figure 3). The interaction effect between zone and experimental condition indicates that exposure also influenced this zone preference. The interaction bar plot (Figure 3) shows an avoidance of the sound source, resulting in increased preference for the zones with the lowest sound pressure levels.
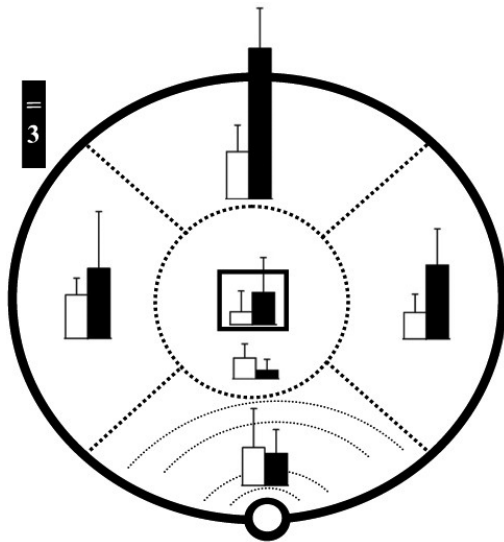
Heart Rate
Typically, during diving, the heart rate was 20 to 30 beats per minute (bpm), rising to 80 to 160 bpm upon surfacing, while prolonged periods at the surface were characterized by periods of intermediate (30 to 60 bpm) bimodal levels of heart rate caused by periods of spontaneous apnea (Figure 2). On average, there was a 30% reduction in heart rate during periods of diving compared to periods at the surface (Figure 6). This pattern

Table 2. Repeated measure ANOVA table; the within-group main effect of *sonar* (on/off) was tested for the dependent variable *surface events*. The different *zones* of the net-cage (Figure 3) are used as the between-factor grouping variable. *Surface events* (number of events per animal in 10 min) are only defined for the experimental groups—not for each individual animal. The main effect of both *sonar* and *zone*, as well as the interaction effects, were all found to be significant. * signifies significant variance. Interaction bar plot is shown in Figure 3.

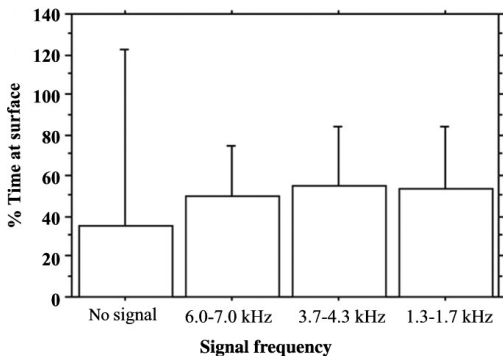| | Dependent variable | |
| | Surface events | |
Factors	F-value	P-value
Sonar	9.3	0.005*
Zone	8.2	<0.0001*
Sonar·Zone	4.5	0.004*

Figure 4. Interaction bar plot for the effect of signal frequency on % time spent at the surface; error bars signify the 95% confidence interval. The ANOVA test implies unequal means (Table 1), but post hoc tests gave no significant differences between no signal control and any of the tested frequencies.

of diving bradycardia did not change during sonar exposure, but the average heart rate increased by 34% during exposure periods compared to the baseline period. Statistical analyses show significant effects on heart rate of both sonar and diving as well as for the interaction between sonar and diving (Table 3). The interaction bar plot (Figure 6) shows that sonar exposure led to a significant 28% increase in heart rate when the animals were at the surface but an insignificant change when the animals were diving (Figure 6).

Discussion

Behaviour
This study showed that young hooded seals started to show active avoidance behaviour in response to 1 to 7 kHz sonar signals transmitted at 10% duty cycle at received sound pressure levels above 160
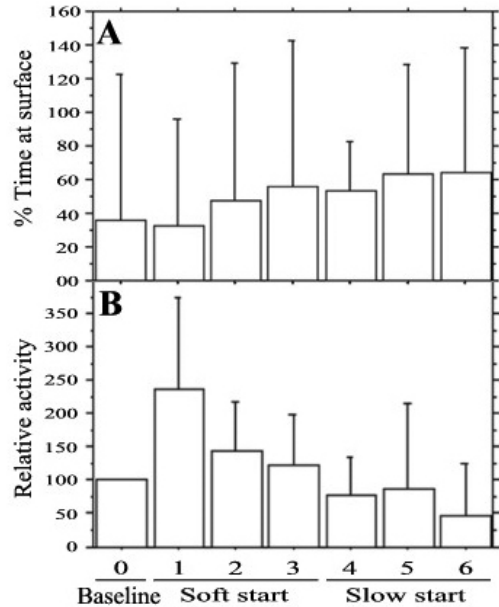

Figure 5. Interaction bar plot for the effect of exposure order on % time spent at the surface (< 1 m depth) (A) and relative activity (B); error bars signify the 95% confidence interval. There is a tendency of an order effect on time spent at the surface (A), which increases with exposure number, but the ANOVA test did not imply unequal means (Table 1). For activity (B), the ANOVA test did imply unequal means (Table 1), and post hoc tests showed significantly higher activity during the first exposure (order 1) compared to both the control period (order 0) and all subsequent exposures (order 2 to 6).

to 170 dB$_{RMS}$ (re 1 µPa). The lack of response to sound below this level is worth noting in view of the fact that this level is well above the hearing threshold (54 to 80 dB [re 1 µPa]) of seals in the frequency range of 1 to 7 kHz (Møhl, 1968; Terhune & Ronald, 1972, 1975; Terhune, 1988; Kastak & Schusterman, 1998; Kastelein et al., 2009a, 2009b). In this context, it is also worth noting that free-ranging elephant seals (*Mirounga angustirostris*) showed no change in diving behaviour when exposed to very low-frequency signals (55 to 95 Hz) at levels up to 137 dB (Costa et al., 2003), and that trained captive sea lions (*Zalophus californianus*) showed avoidance behaviour in response to impulse sounds at levels above 165 to 170 dB$_{RMS}$ (Finneran et al., 2003).

The initial response of our animals was to increase swimming activity at the surface (Figure 5B), apparently to seek out areas of minimum sound pressure level (Figure 3). All animals rapidly adapted to the exposure. Already at the second exposure trial, the increase in swimming activity was no longer evident (Figure 5B), and

Table 3. Repeated measure ANOVA table; the within-group main effect of sonar exposure (sonar on/off) was tested for the dependent variable *heart rate*. The *diving* activity (diving or not diving) was used as a between-factor grouping variable. The main effects of both *sonar* and *diving*, as well as the interaction effect, were all found to be significant. * signifies significant variance. Interaction bar plot is shown in Figure 6.

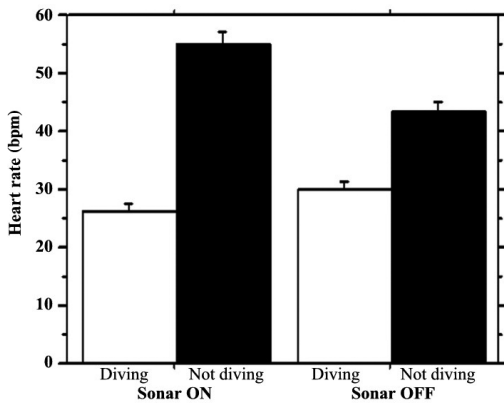| | Dependent variable | |
| | Heart rate | |
Factors	F-value	P-value
Sonar	35.7	< 0.0001*
Diving	454.8	< 0.0001*
Sonar·Diving	73.2	< 0.0001*

Figure 6. Interaction bar plot for the effect of sonar (on/off) and diving (submerged or not) on heart rate; error bars signify the 95% confidence interval. Results of ANOVA tests are summarized in Table 3 and do imply unequal means. Post hoc tests gave significant differences in heart rate between the diving and not diving behavioral state, both during sonar exposures and prior to sonar exposures, and significant differences in heart rate between sonar on and sonar off when the animals were not diving. However, heart rate during diving when sonar was on was not significantly different from heart rate during diving when sonar was off.

the reduction in diving activity and floating with the head out of the water became more conspicuous at every exposure (Figure 5A). Sound conduction pathways for underwater hearing in pinnipeds are not fully understood, but lifting the head out of the water, reduced diving activity, and increased surface time may be a way to reduce exposure to unpleasant or painful sound levels as well as to the risk of hearing injury.

The frequency-modulated up-sweep signals used were chosen because of their operational relevance. Up-sweeps may have a Doppler perception for the animal as if the sound source is rapidly approaching. It cannot be ruled out that the initial response is in part due to this phenomenon and

that a different sonar signal (e.g., a continuous wave or a down-sweep signal) would result in a different response even with the same frequency band. The sonar source used did contain some upper harmonics when transmitting at the maximum source level, particularly at the lower frequency sweep (1 to 2 kHz). However, even the second upper harmonic of the lowest fundamental was attenuated by at least 30 dB; and for the highest fundamental frequencies, the second harmonic was attenuated by at least 50 dB. Since the hearing curve of phocid seals is flat within the band from 200 Hz to at least 40 kHz (e.g., Kastelein et al., 2009a), this would imply that the loudness of the fundamental frequencies would completely dominate the harmonics. It is therefore highly likely that it was the fundamental signals which triggered responses, not harmonics. In fact, our result shows that within the tested band, there is no frequency dependency of the response (Figure 4), which again is not surprising given the flat hearing curve of these animals within this band (e.g., Kastelein et al., 2009a).

In this study, we did not have enough animals at our disposal to be able to rotate the "slow start" and "soft start" exposure protocols on naive animals and thereby properly evaluate if one procedure is significantly different from the other in eliciting avoidance behaviour (Figure 5). It is to be expected, however, that avoidance reactions will be elicited at longer distances in the wild if "slow start" instead of "soft start" is applied since the threshold of avoidance is then reached at a longer distance from the source.

Heart Rate
In the baseline control period, heart rate varied in a normal pattern with diving activity (Figures 2 & 6), while the average (diving and nondiving) heart rate increased by 34% during sonar exposure periods compared to the baseline period. Since the animals spent significantly less time diving during the exposures, most of this increase in heart rate is probably caused by this change in diving behaviour. However, even though the effect of diving is much stronger, a significant effect on heart rate was also found for sonar exposure as well as for the interaction between diving and sonar (Table 3). Heart rate is acknowledged as an indicator of the emotional status of an animal (e.g., Blix et al., 1974), but heart rate also increases with physical (swimming) activity, and in habitually diving animals, it is often dramatically reduced during diving (e.g., Ramirez et al., 2007). It is therefore to be expected, as indeed observed in this study, that when the animals spent more time at the surface in response to sonar exposure, this resulted in increased heart rates (Figure 6). However, our

results also show that when the animals were at the surface, the heart rate was increased during sonar exposure compared to the control period (Table 3). After the initial exploratory response, the activity level during exposure was comparable to or lower than the activity level during the control period (Figure 5), and, thus, there was no increase in physical activity which could explain the increased heart rate during exposure. The increased heart rate at the surface during sonar exposure might therefore indicate emotional activation or discomfort. However, the initial exploratory response followed by rapid behavioural adaptation with passive floating at the surface during sonar exposure indicates that there was no panic. The lack of effect of sonar exposure on heart rate during diving (Table 3; Figure 6) also indicates that despite any emotional activation, normal physiological responses to diving were still intact.

It is also worth noting that while the study animals had the normal profound bradycardia during dives and tachycardia while at the surface between dives, their heart rates when they were floating at the surface showed a bimodal pattern (Figure 2). This pattern, which is particularly conspicuous during sonar exposure because the animals then spent more time at the surface (Figure 2), is typical of pinnipeds at rest, when periods of spontaneous apnea with moderate bradycardia are common (e.g., Pasche & Krog, 1980).

Conclusions
Mid-frequency sonar signals (1 to 7 kHz) transmitted at 10% duty cycle elicited active avoidance behaviour in hooded seals at received sound pressure levels exceeding 160 to 170 dB$_{RMS}$ (re 1 µPa). The behavioural response involved reduced diving and initial swimming away from the sonar source, followed by rapid behavioural adaptation, resulting in passive floating at the surface. No differences were found in behavioural responses in relation to transmitted frequency within the 1 to 7 kHz range tested. Increased heart rate at the surface, which is not explained by increased swimming activity, indicates emotional activation during sonar exposure, but lack of effect of sonar exposure on heart rate during diving indicates that physiological responses to diving remain intact.

Literature Cited

Balcomb, K. C., & Claridge, D. E. (2001). A mass stranding of cetaceans caused by naval sonar in the Bahamas. *Bahamas Journal of Science*, *5*, 2-12.

Blix, A. S., Strømme, S. B., & Ursin, H. (1974). Additional heart rate—An indicator of psychological activation. *Aerospace Medicine*, *45*, 1219-1222.

Costa, D., Crocker, D. E., Gedamke, J., Webb, P. M., Houser, D. S., Blackwell, S. B., et al. (2003). The effect of a low-frequency sound source (acoustic thermometry of the ocean climate) on the diving behavior of juvenile northern elephant seals, *Mirounga angustirostris*. *The Journal of the Acoustical Society of America*, *113*, 1155-1165.

D'Amico, A., & Verboom, V. C. (1998, July). *Report of the bioacoustics panel convened at SACLANTCEN* (SACLANTCEN M-133).

European Union (EU). (2004). *European Parliament resolution on the environmental effects of high-intensity active naval sonars* (B6-0089/04).

Evans, D. L., & England, G. R. (2001). *Joint interim report: Bahamas marine mammal stranding event of 15-16 March 2000*. Washington, DC: National Oceanic and Atmospheric Administration and U.S. Navy.

Fernandez, A., Edwards, J. F., Rodriguez, F., Espinosa de los Monteros, A., Herraez, P., Castro, P., et al. (2005). Gas and fat embolic syndrome involving a mass stranding of beaked whales (family *Ziphiidae*) exposed to anthropogenic sonar signals. *Veterinary Pathology*, *42*, 446-457.

Finneran, J. J., Dear, R., Carder, D. A., & Ridgway, S. A. (2003). Auditory and behavioural responses of California sea lions to single underwater impulses from an arc-gap transducer. *The Journal of the Acoustical Society of America*, *114*, 1667-1677.

Frantzis, A. (1998). Does acoustic testing strand whales? *Nature*, *392*, 29.

International Council for the Exploration of the Sea (ICES). (2005). *Report of the ad-hoc group on the impact of sonar on cetaceans and fish* (ICES CM 2005/ACE:01). Copenhagen, Denmark: Author.

International Union for Conservation of Nature (IUCN). (2004). *Undersea noise pollution* (Resolution RES 3.068). Gland, Switzerland: Author.

International Whaling Commission (IWC). (2004). *Report of the scientific committee 2004*. Cambridge, England: Author.

Jasney, M., Reynolds, J., Horowitz, C., & Wetzler, A. (2005). *Sounding the depth II: The rising toll of sonar, shipping and industrial ocean noise on marine life.* New York: National Resources Defense Council.

Jepson, P. D., Arbelo, M., Deaville, R., Patterson, I. A. P., Castro, P., Baker, J. R., et al. (2003). Gas-bubble lesions in stranded cetaceans. *Nature*, *425*, 575-576.

Joint Nature Conservation Committee (JNCC). (1998). *Guidelines for minimizing acoustic disturbances to marine mammals from seismic surveys.* Aberdeen, UK: Author. Retrieved 6 July 2010 from www.bape.gouv. qc.ca/sections/mandats/sismiques/documents/DD4.pdf.

Kastak, D., & Schusterman, R. J. (1998). Low-frequency amphibious hearing in pinnipeds: Methods, measurements, noise, and ecology. *The Journal of the Acoustical Society of America*, *103*, 2216-2228.

Kastelein, R. A., Wensveen, P., Hoek, L., & Terhune, J. M. (2009a). Underwater hearing sensitivity of harbor seals (*Phoca vitulina*) for narrow noise bands between 0.2 and 80 kHz. *The Journal of the Acoustical Society of America, 126*, 476-483.

Kastelein, R. A., Wensveen, P. J., Hoek, L., Verboom, W. C., & Terhune, J. M. (2009b). Underwater detection of tonal signals between 0.125 and 100 kHz by harbor seals (*Phoca vitulina*). *The Journal of the Acoustical Society of America, 125*, 1222-1229.

Møhl, B. (1968). Auditory sensitivity of the common seal in air and water. *Journal of Auditory Research*, *8*, 27-38.

National Oceanic and Atmospheric Administration (NOAA). (2002). *Regulations governing taking and importing marine mammals: Subpart Q. Taking marine mammals incidental to Navy operations of surveillance towed array sensor system low frequency active sonar* (50 CFR Part 216). Washington, DC: Author.

North Atlantic Treaty Organization Underwater Research Center (NATO URC). (2004). *NATO URC human diver and marine mammal risk mitigation rules* (NATO Underwater Research Center, Staff Instruction 77). La Spezia, Italy: Author.

Pasche, A., & Krog, J. (1980). Heart rate in resting seals on land and in water. *Comparative Biochemistry and Physiology Part A*, *67*, 77-83.

Ramirez, J-M., Folkow, L. P., & Blix, A. S. (2007). Hypoxia tolerance in mammals and birds: From the wilderness to the clinic. *Annual Review of Physiology*, *69*, 113-143.

Scientific Committee on Antarctic Research (SCAR). (2004, May). SCAR report on marine acoustic technology and the Antarctic environment (Information Paper IP 078). *XXVII Antarctic Treaty Consultative Meeting [ATCM]*, Cape Town, South Africa.

Simmonds, M., Dolman, S., & Weilgart, L. (2003). *Oceans of noise.* Chippenham, England: Whale and Dolphin Conservation Society.

Terhune, J. M. (1988). Detection thresholds of a harbour seal to repeated underwater high-frequency, short-duration sinusoidal pulses. *Canadian Journal of Zoology*, *66*, 1578-1582.

Terhune, J. M., & Ronald, K. (1972). The harp seals, *Pagophilus groenlandicus* (Erxleben, 1777): III. The underwater audiogram. *Canadian Journal of Zoology*, *50*, 565-569.

Terhune, J. M., & Ronald, K. (1975). Masked hearing thresholds of ringed seals. *The Journal of the Acoustical Society of America*, *58*, 515-516.