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

Petter H. Kvadsheim,¹ Erik M. Sevaldsen,¹ Lars P. Folkow,² and Arnoldus S. Blix²

¹Norwegian Defence Research Establishment (FFI), Maritime Systems Division, Horten, Norway
E-mail: phk@ffi.no
²Department of Arctic Biology, University of Tromsø, 9037 Tromsø, Norway

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 harengus) 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 omnidirectionally 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 m³ (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).
Seals and Sonar

**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 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 up-sweep (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
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 \( \leq 1 \) 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\(_{1\mu Pa \text{ re } 1 m}\) 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
The 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 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.

### 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.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Activity</th>
<th>Time at surface</th>
<th>Dive frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-value</td>
<td>P-value</td>
<td>F-value</td>
</tr>
<tr>
<td>Exposure order</td>
<td>4.9</td>
<td>0.04*</td>
<td>2.1</td>
</tr>
<tr>
<td>Signal type</td>
<td>1.9</td>
<td>0.24</td>
<td>10.1</td>
</tr>
</tbody>
</table>

### 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
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 the interaction effects, were all found to be significant. * signifies significant variance. Interaction bar plot is shown in Figure 3.

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.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Surface events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
<td>F-value</td>
</tr>
<tr>
<td>Sonar</td>
<td>9.3</td>
</tr>
<tr>
<td>Zone</td>
<td>8.2</td>
</tr>
<tr>
<td>Sonar·Zone</td>
<td>4.5</td>
</tr>
</tbody>
</table>

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 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
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

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
<td>F-value</td>
</tr>
<tr>
<td>Sonar</td>
<td>35.7</td>
</tr>
<tr>
<td>Diving</td>
<td>454.8</td>
</tr>
<tr>
<td>Sonar·Diving</td>
<td>73.2</td>
</tr>
</tbody>
</table>

* signifies significant variance. Interaction bar plot is shown in Figure 6.
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_{1ms} (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.

Acknowledgments
We thank Commander Geir Morten Bentzen and his crew at the Olavsvern Naval Base in Tromsø for logistical support; M. Motzfeldt, J. Ness, and H. Lian for expert care of the animals during the experiments; Ingebrit Gausland for sharing his ideas of “slow start” with us; Kongsberg Defense and Aerospace (KDA) for letting us use their L50 power amplifier; DLAB/SINTEF for supplying the transducer used during the tests; and Professor Lars Walløe, University of Oslo, for advice during the preparation of the manuscript. This project was financially supported by the Royal Norwegian Navy, the Norwegian Ministry of Defence, and the University of Tromsø.

Literature Cited


