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Auditory studies on marine mammals

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SUMMARY

The diversity and amount of anthropogenic activities in the German waters of the North and Baltic Seas is high compared to other marine areas. Numerous of these activities are linked to the emission of intense sound into the marine environment. Intense underwater sound has been identified as a potential threat especially for marine mammals, which are represented in German waters by the harbor porpoise, a small whale species, the harbor seal and the gray seal. Concern about sound-induced effects on these species was raised especially with regard to the construction of offshore wind turbines and the use of seismic airguns for marine geophysical surveys conducted for hydrocarbon exploration.

The potential effects induced by intense sounds can range from an increased level of stress, behavioral reactions, acoustic masking of communication or other biologically relevant signals to a temporary or permanent impairment of the hearing system. Hearing is an important sensory modality in all three species, and especially harbor porpoises rely on their hearing for survival. Several auditory studies were conducted on harbor porpoises and harbor seals to provide a scientific basis for assessing the scale of potential sound-induced effects. In addition, a method to reduce the sound immissions to harbor porpoises was developed and tested.

To meet the objectives of the studies, a method was required which allowed conducting comparable auditory measurements in marine mammals both in the wild and under controlled conditions in human care. The measurement of auditory evoked potentials (AEPs) appeared to be the most suitable method. This neurophysiological method was successfully tested and applied to collect auditory data on sub-adult wild harbor seals on a sandbank and harbor seal pups in a seal center. The data show that these animals were apparently unaffected by noise as their hearing thresholds were comparable with published data. Thus, these groups can be considered as a control group for a future long-time monitoring of the auditory health status of harbor seals. A similar study was conducted on harbor porpoises in a rehabilitation center. The results of these auditory measurements show that this method is suitable for auditory measurements in wild animals. Moreover, the auditory thresholds determined in this study can be used as a data basis for comparison.

The operational sound of offshore wind turbines was considered potentially causing an auditory masking effect in harbor porpoises. A dedicated AEP study under controlled acoustical conditions with a trained animal revealed that operational sounds emitted by a 2 MW wind turbine would only mask the acoustic perception of harbor porpoises at close ranges.

Intense impulsive sounds were tested for their potential auditory effect in a harbor porpoises in another AEP study. The animal's auditory tolerance was determined by systematically increasing the received levels of a fatiguing sound stimulus and repeatedly testing the animal's auditory sensitivity. At a sound pressure level of above 200 dB re 1 μ Pa and a sound energy of 164 dB re 1 μ Pa²·s the animal's hearing threshold shifted temporarily, thus providing the first scientific basis for a noise exposure criterion for this species.

Summary

An air bubble curtain was constructed to reduce strong avoidance behavior shown by three harbor porpoises in a facility near a pile driving site. This system led to reduction in received sound on average by 16 dB. The animals returned to their normal behavior when the air bubble curtain was activated, thus proving the efficiency of the air bubble curtain.

Such an air bubble curtain is one example of a variety of potential measures to minimize or mitigate sound-related effects. Like most other mitigation measures, the air bubble curtain can still be optimized. As a general principle, the best available techniques should always be used and depending on the situation, the best combination of all suitable measures should be applied to achieve the strongest mitigation effects.

The increasing ecological awareness about anthropogenic noise as a problem for the marine environment and economic considerations may lead to reduced sound emissions from some anthropogenic activities. Those activities whose noise emissions remain at high levels or levels considered detrimental for the marine environment may be limited by national or international regulations in the future.

ZUSAMMENFASSUNG

Die zunehmende Nutzung der Meere durch den Menschen geht mit einem steigenden Eintrag von Lärm in die marine Umwelt einher. Die Nord- und Ostsee unterliegen einer besonders starken anthropogenen Nutzung, so dass von einem erhöhten Lärmpegel unter Wasser auszugehen ist. Intensiver Schall stellt eine potentielle Gefährdung für marine Lebewesen, vor allem für marine Säugetiere dar. Diese sind in den deutschen Gewässern der Nord- und Ostsee durch drei Arten vertreten: den Schweinswal, eine kleine Zahnwalart, den gemeinen Seehund und die Kegelrobbe. Für alle drei Arten, aber besonders die Schweinswale ist ein funktionierendes Gehör von vitaler Bedeutung. Die mit der Errichtung und dem Betrieb von Offshore-Windenergieanlagen sowie dem Einsatz von seismischen Airguns einhergehenden Schallemissionen geben Anlass für eine mögliche akustische Beeinträchtigung dieser Arten.

Eine Beschallung kann bei den marinen Säugetieren zu einem erhöhten Stresspegel, Verhaltensreaktionen, der akustischen Maskierung von Kommunikationslauten oder bei hohen Schallpegeln zu einer zeitweiligen oder dauerhaften Beeinträchtigung des Gehörs führen. Um die möglichen Auswirkungen anthropogener Schallquellen auf die marinen Säugetiere besser beurteilen zu können, wurden mehrere auditorische Studien an Schweinswalen und Seehunden durchgeführt.

Um vergleichbare Untersuchungen an freilebenden Tieren und an Tieren in Gefangenschaft durchführen zu können, wurde die Messung auditorisch evozierter Potenziale (AEP Methode) gewählt. Diese elektrophysiologische Messmethode wurde an subadulten freilebenden Seehunden sowie an Heulern in einer Seehundstation erfolgreich getestet und für auditorische Messungen angewandt. Die ermittelten Hörschwellenwerte sind vergleichbar mit veröffentlichten Werten, so dass davon auszugehen ist, dass die untersuchten Tiere keine Hörschäden aufwiesen. Die beiden untersuchten Altersgruppen können somit beispielsweise als unbelastete Kontrollgruppe für ein zukünftiges akustisches Gesundheits-Monitoring der Population der Nord- und Ostsee herangezogen werden. Eine vergleichbare Studie wurde an mehreren Schweinswalen in einer Rehabilitationseinrichtung durchgeführt.

In einer der Studien wurde der akustische Maskierungseffekt der Betriebsgeräusche von Offshore-Windenergieanlagen untersucht. Es bestand Grund zu der Annahme, dass diese Geräusche die akustische Wahrnehmung der Schweinswale einschränken könnte. Die Ergebnisse zeigen jedoch, dass die Schallemissionen der derzeit vorhandenen 2 MW-Anlagen bei Schweinswalen lediglich im direkten Nahbereich der Anlagen zu einer akustischen Maskierung von Geräuschen führen.

Die akustische Belastbarkeit von Schweinswalen gegenüber Schallimpulsen wurde in einer weiteren AEP-Studie untersucht. Dabei wurde ein Schweinswal wiederholt Schallimpulsen mit einem systematisch gesteigerten Schalldruckpegel ausgesetzt und sowohl davor als auch danach jeweils audiometrisch untersucht. Ab einem Spitzenpegel von 200 dB re 1 μ Pa und einer Schallenergie von 164 dB re 1 μ Pa²·s konnte bei dem Tier eine zeitweilige Verschlechterung der Hörschwelle festgestellt werden. Diese Werte ermöglichen

erstmalig eine wissenschaftlich fundierte Festlegung eines akustischen Belastungsgrenzwertes für Schweinswale.

Die Entwicklung und Erprobung eines Luftblasenschleiers zur Schallminderung unter Wasser war Gegenstand einer weiteren Studie. Dieses System wurde eingesetzt, da Schweinswale in einer Einrichtung starke Vermeidungsreaktionen zeigten, als in ihrer Nähe mit Rammarbeiten im Wasser begonnen wurde. Durch den Luftblasenschleier konnte der Schalldruckpegel der Rammimpulse im Becken der Tiere um durchschnittlich 16 dB reduziert werden. Die Wirksamkeit einer derartigen Schallreduktion zeigt sich außerdem darin, dass die Tiere wieder zu ihrem normalen Verhalten zurückkehrten.

Der Luftblasenschleier ist nur eine von zahlreichen möglichen Maßnahmen zur Vermeidung oder Verminderung von schallbedingten Auswirkungen. Wie bei zahlreichen anderen Minderungsmaßnahmen, so kann auch der Luftblasenschleier noch optimiert werden. Generell sollten die Maßnahmen immer nach dem besten verfügbaren Stand der Technik eingesetzt werden. Darüber hinaus sollte die optimale Kombination aller sinnvoll einzusetzender Verfahren gewählt werden, um den größtmöglichen Minimierungseffekt erzielen zu können.

Aufgrund des wachsenden ökologischen Bewußtseins hinsichtlich der Auswirkungen von Schall auf den marinen Lebensraum sowie aus ökonomischen Beweggründen ist eine Reduzierung der Schallemissionen zumindest bei einigen Schallquellen wahrscheinlich. Andere Schallquellen, deren Emissionen unverändert hoch bleiben und als nicht vertretbar angesehen werden müssen, könnten zukünftig durch nationale oder internationale Vorschriften begrenzt werden.

INTRODUCTION

Humans began to use the marine environment probably several thousand years ago (Bednarik and Kuckenbug, 2001). Starting with fishing and the most simple forms of transportation the diversity of human activities and their intensity increased steadily; with an exponential rise since the beginning of industrialization. All of these so-called anthropogenic activities are today linked to the emission of sound into the water (Richardson *et al.*, 1995), both purposefully and unintentionally.

Over the past decades this has led to a doubling of the underwater noise level per decade in highly industrialized areas (Hildebrand, 2004). This increase has mainly been attributed to an increase in commercial shipping (Cato and McCauley, 2002; Zakarauskas, 1986; Curtis *et al.*, 1999; Andrew *et al.*, 2002; Heitmeyer *et al.*, 2004; Hildebrand, 2004; Ross, 2005; McDonald *et al.*, 2006; Hatch *et al.*, 2008). Commercial shipping lines are also important life-lines for the highly industrialized European countries abutting the North Sea and Baltic Sea. There is growing traffic between European States and other parts of the world, and especially the North Sea is one of the most frequently traversed sea territories in the world (OSPAR Commission, 2000). Even though no long-term data sets of ambient sound level are available, the average noise level in the North Sea and Baltic Sea likely increased at least to the same extent as in other parts of the world's oceans.

An increasing diversity and amount of other anthropogenic activities in the North Sea and Baltic Sea also produce a substantial amount of sound thus reinforcing this trend even more. The most relevant contributors to anthropogenic ambient noise besides shipping are marine geophysical surveys conducted for hydrocarbon exploration, offshore construction (e.g. pile driving), industrial activities (e.g. aggregate extraction, drilling), defense-related activities, and research (Richardson *et al.*, 1995; OSPAR Commission, 2000). The emitted sound can be high-intensity and acute, as well as lower-level and chronic and is dominated by low frequencies. Some activities, such as seismic surveys, pile driving, sonars and explosions cause impulsive sounds which can comprise frequencies in the ultrasonic region (>20 kHz) at significant levels. At least at close ranges shipping also contributes to this ultrasonic frequency band (Arveson and Venditis, 2000; Aguilar Soto *et al.*, 2006).

Currently another large-scale anthropogenic activity is envisaged for these areas. Large numbers of wind turbines are planned to be built in offshore areas of the North and Baltic Seas as part of the efforts to increase the use of renewable energies and thus meet the goals of the Kyoto protocol and its successors. For the German waters alone, permits for more than 1,500 wind turbines were already granted by the BSH (2008). While this effort *per se* can be regarded as beneficial also for the marine environment (Harley *et al.*, 2006), the approach to its implementation raises concern about potential adverse effects on the marine environment. One of the key issues is the sound emitted into the water column during the construction of the turbines and the sound's effects on marine mammals. The most common method to install the pile foundations for these wind turbines is impact pile driving. The installation of a single foundation involves several thousand strokes with each impact peak

reaching a source level of 230 dB re 1 μ Pa and more (Parvin *et al.*, 2006) thus contributing considerably to the noise pollution in the marine environment.

But the marine environment in its natural state is also not as quiet as one might think. Numerous non-biological sound sources (waves, precipitation, earthquakes, etc.) exist which constantly create a natural underwater background noise of varying intensity and composition (Richardson *et al.*, 1995). Besides these physical or geological processes, a variety of biological sound sources also contribute to the acoustic scene underwater. From “snapping” shrimp to “singing” whales, the emitted sounds comprise unintentional mechanical sounds as well as purposefully produced vocal sounds. Both can, actively or passively, intended or unintended, contain important information for a receiver.

Marine mammals evolved in the presence of this diverse natural sound environment and their hearing sensitivity is well adapted to perceiving signals biologically significant to them. Pinnipeds (seals) and cetaceans (whales, dolphins and porpoises) have adapted their sensory systems to an entirely aquatic (cetacean) or amphibious (pinniped) life-style. Pinnipeds and cetaceans produce and receive sound over a great range of frequencies for use in communication, predator avoidance and to interact with their environment. Some toothed whale (odontocete) species have the capability to use echolocation for foraging and orientation in their underwater environment (Tyack, 1997; Au, 2000; Tyack and Clark, 2000). For these species sound became the most important sensory modality, and they rely on hearing for survival.

Of all 124 marine mammal species (Rice, 1998) there are only three species resident in the German waters of the North and Baltic Seas: the harbor seal (*Phoca vitulina*) and the gray seal (*Halichoerus grypus*) as well as the harbor porpoise (*Phocoena phocoena*), a small toothed whale species. These three species share, as far as information is available, the sophisticated and very acute sense of hearing in marine mammals.

In-air sound has been identified as a potential hazard for humans as it may cause effects ranging from stress to noise-induced hearing loss (NIHL) and has widely been recognized as an environmental hazard (Lataye and Campo, 1996). Damage-risk criteria (DRC; e.g. Eldred *et al.*, 1955, cf. from Lataye and Campo, 1996) have served as basis for protective measures in the workplace as well as in the public space for decades (ISO, 1981; BMU, 1998). Recently underwater noise became an issue within legislative bodies (e.g. the European Union) only recently. The EU Marine Strategy Framework Directive aims to establish a Good Environmental Status (GES) in the marine environment. A prerequisite for this is that the “*Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment*” (EU, 2008).

Sound in general can have diverse negative effects on marine mammals. As in terrestrial mammals (including humans), sound can cause acute or chronic stress (humans: Chrousos and Gold, 1992; marine mammals: Fair and Becker, 2000; Reeder and Kramer, 2005; Martineau, 2007; Nowacek *et al.*, 2007; Wright *et al.*, 2007), it may impede the perception of other biologically meaningful sounds (“masking”) (Richardson *et al.*, 1995; NRC, 2003; Janik, 2005; Madsen *et al.*, 2006), it can trigger behavioral reactions (NRC 2005; Southall *et al.*,

2007; Nowacek *et al.*, 2007), and even lead to direct physiological or physical impairment and injury (Ketten *et al.*, 1993; Finneran *et al.*, 2002, 2005; Nachtigall *et al.*, 2003, 2004; Kastak *et al.*, 2008).

Marine mammals are almost inaccessible for research due to their aquatic lifestyle. Consequently, not much is known about their acoustic sensitivity and tolerance. Noise exposure criteria have been defined in several countries (e.g. UBA, 2003; Ketten and Finneran, 2004; Southall *et al.*, 2007), but these values rely mainly on extrapolations from a few studies conducted on a limited number of different species.

Based on the available information of the sound emissions from pile driving and other intense sound sources, on the one hand, and known effects of intense sound on terrestrial as well as some marine mammals, on the other hand, it can be hypothesized that both seal species as well as harbor porpoises will be able to perceive these anthropogenic sound emissions and are likely to be impacted by them to varying degrees.

The resulting questions for the studies were:

- Are seals originating from the North Sea already affected by the sound level in their environment?
- Is anthropogenic sound likely to interfere with the animals' communication?
- How tolerant is their hearing to exposure to the most common type of intense sound?
- Are there practical acoustic mitigation measures?

To address these questions it was necessary to choose and develop a technique allowing comparable auditory testing of captive and wild animals. So far, captive animals were most often behaviorally trained over a longer period to participate in psychophysical auditory testing. However, it was evident that access to wild animals for auditory testing would be limited to several hours. Therefore, the electrophysiological method of measuring auditory evoked potentials (AEPs) was chosen for studies on both captive and wild animals, thus enabling direct comparison of the results. This technique had to be established for the specific purpose of the studies, and different study designs had to be developed in order to address the requirements of each study.

A brief introduction to the AEP method is given below, a detailed description of the methodology and the specific methodological approaches chosen under each study condition can be found in the individual chapters.

The data collected throughout the studies compiled in this dissertation are intended to:

- form a basis for assessing the auditory sensitivity of marine mammals in German waters;
- determine potential effects of offshore wind turbine-related sound emissions;
- determine species specific noise exposure criteria for harbor porpoises; and
- support defining sensible mitigation measures.

The studies presented in this dissertation focus on the harbor porpoise and the harbor seal. Due to their low numbers in German waters and relative inaccessibility the gray seal could not be included into the dedicated auditory studies. However, a single wild individual was tested for its auditory sensitivity and the resulting data is presented in the final part of this dissertation.

NATURAL HISTORY OF THE HARBOR PORPOISE (*PHOCOENA PHOCOENA*,
LINNAEUS 1758)

(with special emphasis on acoustic aspects)

The harbor porpoise (*Phocoena phocoena*, Linnaeus 1758), a small toothed whale is distributed throughout most coastal waters of the Northern hemisphere and represented with a subspecies, *P. p. phocoena*, in the North Atlantic (Rice, 1998). In German waters these small whales can be found throughout the year both in the North Sea and Baltic Sea. Harbor porpoises predominantly occur solitary or in small groups, e.g. mother-calf pairs. As revealed by a dedicated “census” in German waters, the average group size in German waters was determined to be between 1.17 and 1.8 (Scheidat *et al.*, 2004; Siebert *et al.*, 2006).

As all cetaceans they are fully aquatic animals, remain submerged for most of their time and hence are notoriously difficult to study. At sea harbor porpoises can be identified by their dark dorsal coloration and the triangular-shaped dorsal fin when they surface. They have a spine-shaped body and homodont spate-shaped teeth but no distinct beaks like dolphins. As all whale species harbor porpoises have no external ear and only dysfunctional remnants of the outer ear canals can be located ventrocaudad from the eyes. With an average weight between 45 and 73 kg (males/females) and an average adult body length of 152 cm to 162 cm (males/females) (Benke *et al.*, 1998) they are one of the smallest whale species (figure 1 and 2).

Harbor porpoises are protected under several international treaties such as the “*Convention for the Conservation of Migratory Species of Wild Animals*” (“*Bonn Agreement*”, 1979) and Annex II of the “*Bern Convention*” (1982). They are listed in Annex II and IV of the European Union’s “*Directive on the Conservation of Natural Habitats and Wild Fauna and Flora*” (“*Habitats Directive*”; EU Council, 1992). In Europe, this species is also protected under the “*Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas*” (ASCOBANS, 1992) ratified by Germany in 1994. With an estimated global abundance of 700,000 individuals, its international Red List status was recently determined as “least concern” by the IUCN (2008). Genetic and morphological investigations showed that harbor porpoises from the German North Sea belong to a single subpopulation (southern and central North Sea), while two distinct subpopulations can be found in in the Baltic Sea, one in the western Baltic (including Kattegat, inner Danish and German waters) and one in the eastern Baltic (Tiedemann *et al.*, 1996; IWC, 2000; Huggenberger *et al.*, 2002). In the German Red List, the population in the German North Sea is listed as “vulnerable” (von Nordheim and Merck, 1995), and in both lists the Baltic harbor porpoise population (treated as one population) is identified as “critically endangered” (Merck and von Nordheim, 1996; IUCN, 2008).



Figures 1 and 2. Natural habitus of a harbor porpoise with its typical spine shaped body. The dorsal dark gray coloration and small triangular dorsal fin are well distinguishable. © Klaus Lucke / Fjord & Bælt.

Harbor porpoises reach sexual maturity at the age of three to four years (Benke *et al.*, 1998; Lockyer and Kinze, 2003), and females give birth to one calf after approximately 10 months of gestation in June/July. The lactation period is approximately 10 months, with newborns beginning to feed on fish at approximately 5-6 months of age (Schulze, 1996). Harbor porpoises have a wide food spectrum (Bjørge and Tolley, 2002; Gilles *et al.*, 2008). They show a preference for gadoids and clupeids, but forage also for demersal fish such as gobies and flatfish.

A dedicated line transect survey was conducted in 1994 to estimate the abundance and distribution of harbor porpoises and other small cetaceans in the North Sea and adjacent waters (Hammond *et al.*, 2002). The counts resulted in a total of 341,366 harbor porpoises (with a 95% confidence interval: 160,000-449,000). In 2005, this effort was repeated, yielding comparable counts but with shifted hotspots in their distribution.

Harbor porpoises have the ability to use sound for passive listening as well as active echolocation (Busnel and Dzedzic, 1967) where they probe their environment with sound (Tyack, 1997). They produce short high-frequency, narrow-band impulses ("clicks") (Hansen, 2007; Villadsgaard *et al.*, 2007) in their nasal passage (Amundin *et al.*, 1988; Cranford *et al.*, 1996), emit them via their melon and analyze the echoes returning from objects in their

surroundings, thus gaining an “acoustic image” of their environment. The main energy of their echolocation signals is centred in the ultrasonic range of 110-140 kHz (Verboom and Kastelein, 1995), an extremely high frequency range even among toothed whales. Harbor porpoises have therefore been classified as “high-frequency” cetaceans (Southall *et al.*, 2007).

They use this acoustic capability to spatially navigate in their underwater environment (Verfuß *et al.*, 2005), to locate and identify suitable prey and probably even to communicate (Verboom and Kastelein, 1995).

Their hearing abilities have been investigated in several studies (Andersen, 1970; Popov *et al.*, 1986; Bibikov, 1992; Kastelein *et al.*, 2002). These studies showed that their range of best hearing (defined as 10 dB within the lowest threshold) is 16 to 140 kHz. As harbor porpoises are predominantly feeding on demersal or benthic prey, their ability to detect and discriminate targets in the water column or buried in sand were also studied (Kastelein *et al.*, 1997, 1999).

It remains unclear if harbor porpoises use acoustic signals for communication at all. They have never been proven to produce frequency-modulated whistles or other sounds related to communication (Hansen, 2007). In contrast to several delphinid species which use whistles to communicate (Caldwell *et al.*, 1973; Janik, 2005) and likely to coordinate the behavior in larger groups, active communication might have no ecological benefit for a predominantly solitary living species as the harbor porpoise.

Harbor porpoises are considered to be affected by several adverse factors, mainly of anthropogenic origin. While they have no natural predator in the southern North Sea, they are affected by chemical pollution (Jepson *et al.*, 1999; Siebert *et al.*, 1999). In addition, food depletion is likely contributing to habitat degradation (Reijnders, 1992). The most important threat, however, is incidental bycatch in bottom set nets (Vinther, 1999; Vinther and Larsen, 2004). According to their study of Danish fisheries, an estimated 5,591 animals died due to bycatch between 1987 to 2001. As air breathing mammals, harbor porpoises drown once entangled in the nets.

NATURAL HISTORY OF THE HARBOR SEAL (*PHOCA VITULINA*,
LINNAEUS 1758)

(with special emphasis on acoustic aspects)

The harbor seal (*Phoca vitulina*, Linnaeus 1758) is found throughout the coastal waters of the northern hemisphere (King, 1983; Reijnders *et al.*, 1997; Burns, 2002). One of their five subspecies, *P. v. vitulina*, (the eastern Atlantic harbor seal) is distributed along the coastlines of the eastern North Atlantic. The harbor seals in German North Sea waters belong to a population inhabiting the south-eastern North Sea. The species also occurs in the German Baltic Sea but are not resident in these waters. In the German North Sea the harbor seal breeds in colonies found during low tide on sandbanks and occasionally on beaches in the Wadden Sea (Reijnders *et al.*, 2005). This species was hunted in Germany until 1976 (Reijnders, 1983). Since then their numbers have increased in the entire Wadden Sea from less than 4,000 to 20,250 individuals in 2008, despite two virus-epizootics in 1988 and 2002 (TSEG, 2008). With a global population of 350,000 to 500,000 harbor seals, their Red List status is “least concern” (IUCN, 2008). Recent counts of the subspecies *P. v. vitulina* revealed a regional population size of approximately 100,000 animals (ICES, 2008).

Harbor seals show sexual dimorphism with male seals reaching body lengths of 175 cm and females reaching a body length of 150 cm. Distinguishing features are irregular grey to brownish grey coloration with black spots, and a relatively round shaped head (figure 3). They venture onto land for parturition and nursing as well as for molting (Drescher, 1979). Harbor seals reproduce annually and give birth in the German Wadden Sea from May to July, with a peak of births in June (Drescher, 1979). They nurse their offspring for about four weeks. At sea, harbor seals are predominantly solitary animals. They show a certain degree of site fidelity, but large-scale movements of individual seals to their feedings grounds have been monitored as well (Reijnders *et al.*, 2005; Adelung *et al.*, 2004). Harbor seals are generally opportunistic feeders (Burns, 2002). In the early 2000s, the diet of harbor seals from the German part of the Wadden Sea consisted predominantly of gadoid and flatfish species (Gilles *et al.*, 2008), whereas in the Dutch part of the Wadden Sea mainly flatfish species were consumed and gadoids were only a minor component (Brasseur *et al.*, in press).



Figure 3. Harbor seal hauled-out on a sandbank. © Ilka Hasselmeier

The most important sensory modality for harbor seals is their tactile sense (to forage and navigate based on hydrodynamic cues; Dehnhardt *et al.*, 2001; Schusterman *et al.*, 2004). However, these animals also evolved an acute sense of hearing for aerial and underwater sounds.

Like other phocid species harbor seals use acoustic signals to interact with their environment, including con-specifics (Schusterman and Van Parijs, 2003). This involves vocalization (active) and listening (passive) for communication purposes both in air and underwater. Mother-pup bonds and contact are largely maintained via acoustic signals produced by the pups. In harbor seals, sound is also a vital element in other, broader social communications, including intraspecific competition and intersexual interactions (Schusterman *et al.*, 2001). They rarely produce sounds in air (exceptions: pups – occasional “howling” and guttural threats). In contrast to their behavior on land, they develop complex underwater vocal repertoires. Recent studies have shown that males are vocalizing underwater during the mating season (Van Parijs *et al.*, 1999). This kind of vocalization may be used to attract females or in the context of territorial and competitive behavior towards other males (Hanggi and Schusterman, 1994; Van Parijs *et al.*, 1999, 2000a, 2000b; Bjørgesæter *et al.*, 2004). The extent to which female harbor seals use sound actively, e.g. for mother-infant recognition or individual recognition, remains unclear. However, it is evidently essential in sea lions returning from the sea to find and suckle their offspring in dense colonies.

Acoustic cues are also likely to be used by harbor seals to avoid predators. They may also identify the location of prey from sound emitted by e.g. soniferous fish.

In adaptation to their amphibious lifestyle, harbor seals show an acute hearing sensitivity both for in-air and underwater sound. A number of behavioral psychophysical and

electrophysiological hearing tests were conducted on captive harbor seals over the past decades, both in air and underwater (Møhl, 1968; Terhune, 1991; Kastak and Schusterman, 1998; Kastak *et al.*, 1999; Holt *et al.*, 2001; Wolski *et al.*, 2003; Kastak *et al.*, 2005; Reichmuth *et al.*, 2007). The studies revealed that harbor seals have a functional hearing range from 0.1 kHz to 30 kHz in air and from below 0.1 kHz to at least 128 kHz for underwater sounds.

The main anthropogenic threats for harbor seals are chemical pollution (Reijnders, 1986), and disturbance in several forms (Thiel *et al.*, 1992; Reijnders *et al.*, 2005). Two phocine distemper virus epizootics also caused massive mortalities in the harbor seal population, namely approximately 60% in 1988 and 50% in 2002 (Reijnders and Brasseur, 2003; Härkönen *et al.*, 2006). Immunological investigations show that chemical pollutants can suppress immunefunction in captive harbor seals (De Swart *et al.*, 1996; Ross *et al.*, 1996). This may have aggravated the extent of the mortality toll. However, it is noted that in a population naïve to a certain virus, the effect of a newly introduced virus will be similar to what was observed then. This is supported by the fact that the mortality in 2002 was nearly 10% lower than in 1988, and this is attributed to the number of animals that survived the first epizootic (Reijnders *et al.*, 2003). Chemical analyzes of tissue samples from harbor seals of the second epizootic showed that the levels of organochlorines in adult seals were only half of those found in 1988 (Reijnders and Simmonds, 2003; Reijnders *et al.*, 2009; Weijs *et al.*, 2009).

AUDITORY EVOKED POTENTIAL METHOD

The auditory evoked potential (AEP) method enables rapid measurements of auditory sensitivity even in situations in which the subject is unwilling or unable to participate in common behavioral testing. Since it is non-invasive (in the way it was used in these studies) and requires only minimal (active) cooperation in human clinical use, it has been widely adopted in human patients and is also used for screening of newborns (Hall, 2006).

It is based on the presentation of acoustic stimuli which will generate a synchronous discharge of multiple neurons in the auditory complex upon perception of these stimuli. These AEPs form an electric field in the body and can be measured as potentials from electrodes placed either as sub-dermal needles or with surface electrodes on the surface of the subject's skin (Lenarz, 1993; Goldstein and Aldrich, 1999). To detect these relatively small potentials reliably within the multitude of non-auditory neuronal signals, the acoustic stimuli are repeatedly presented to the subject and the measured potentials are averaged coherently. Thereby, all non-acoustic or incoherent neuronal potentials are reduced or completely eliminated. Depending on the amount of non-auditory potentials, e.g. potentials originating from muscular activity (myogenic potentials), the number of averages has to be adjusted accordingly to eliminate those "artefacts" from the auditory measurements.

The type of acoustic stimulus to be used depends on the objective of the measurement. For diagnostic purposes, broadband clicks can be used (Goldstein and Aldrich, 1999). They elicit neuronal responses from great portions of the basilar membrane, where the mechanical sound energy is translated into neuronal information, and result in larger AEP amplitudes than other stimuli. AEPs are a summation of several neuronal signals generated by different components along the auditory system. Threshold measurements are based on neuronal potentials originating in the cochlea and the brainstem (therefore termed auditory brainstem responses, ABR). Based on latencies and amplitudes of the neuronal potentials, it is possible to assess sensory or neuronal pathologies within the hearing system (Goldstein and Aldrich, 1999). Short sinusoidal signals (tone pips), have a narrower bandwidth, resulting in a frequency specific stimulation of sensory hair cells along the basilar membrane. These responses reflect the subject's auditory sensitivity at a particular frequency range and can be used to determine the auditory thresholds (Supin *et al.*, 2001).

A refined methodological approach is based on the use of rhythmic sound modulations (Supin *et al.*, 2001). By sinusoidally modulating the amplitude of a carrier tone or sound pulse sequence (tone burst), it is possible to elicit a neuronal response which includes a specific frequency component equivalent to the modulation frequency used. This effect occurs because the auditory system is responding to the carrier frequency but the neurons are firing at the rate of the modulation frequency (Au and Hastings, 2008), an effect called envelope-following response (EFR). An alternative method is the presentation of continuous amplitude-modulated sine waves which elicits a continuous and frequency-specific brainstem response (in humans: Lins and Picton, 1995; John *et al.*, 1998). This method of measuring the auditory steady state response (ASSR) has now been successfully tested in toothed whales (Dolphin 1996; Finneran and Houser, 2007).

PHYSICS OF UNDERWATER SOUND

Sound travels as a pressure wave through a medium such as air or water. Its primary properties are amplitude, phase and frequency. The latter is the rate of oscillation, measured in Hertz (Hz). The auditory range in healthy humans reaches from 20 Hz to 20 kHz (Crocker, 1998). Sound with frequencies below 20 Hz is referred to as infrasound, at frequencies above 20 kHz as ultrasound. Baleen whales, for example, are known to produce sounds in the infrasonic region while toothed whales like the harbor porpoise emit signals covering a frequency range of up to 160 kHz.

The parameter commonly used to describe sound intensity is sound pressure, which is defined as

$$\begin{aligned} \text{dB SPL} &= 10 \log (p_m^2/p_r^2) \\ &= 20 \log (p_m/p_r) \end{aligned}$$

with sound pressure level (SPL), a measured pressure (p_m) and a reference pressure (p_r).

The human ear, which is designed as a pressure receiver, can perceive sound at pressures ranging from 20 micro Pascal (μPa) to 20 Pa (i.e. six orders of magnitude) without being harmed. To increase the resolution near the hearing threshold (i.e. at the lower pressure levels), the signal processing within the human systems is based on a logarithmic gradient. With reference to this and to conveniently express values over this large range, the non-dimensional logarithmic “Decibel” (dB) scale is used. Decibel is an abstract logarithmic scale which has to be related to a reference pressure to be informative. For in-air sound this reference pressure is 20 μPa (i.e. the lowest sound pressure level humans can detect), in underwater acoustics the conventional reference pressure is 1 μPa . The logarithmic definition of the decibel scale implies that a doubling in sound pressure corresponds to a 6 dB increase in sound pressure level.

To compare sound pressure levels received in air and underwater, the difference in density between the media as well as the different reference values have to be taken into account. The characteristic impedance of water is about 3600 times that of air; which equals $10 \log (3600) = 35.5$ dB. This accounts for a difference of sound pressure levels in both media of 35.5 dB and another 26 dB have to be added due to the difference in reference pressures. To be perceived at even levels by a hearing system equally sensitive to sound in both media, the sound pressure level of underwater signals would therefore have to be 61.5 dB higher than for those in air (Ketten, 1998). However, direct comparisons between in-air and underwater sound with regard to acoustic reception are speculative and should be considered cautiously.

Different measuring methods and metrics exist to quantify the amplitude and energy of the acoustic signal (Richardson *et al.* 1995; McCauley *et al.*, 2000; Madsen, 2005). Throughout this dissertation, several metrics have been used depending on what kind of acoustic signals and which of their acoustic properties were described. A definition of all units and terms used can be found in the individual chapters and the glossary.

EFFECTS OF NOISE

It should be noted that the terms “sound” and “noise” are not synonymous. While sound is referring to any kind of acoustic energy, noise refers to the sound unwanted by the perceiving subject. Signals on the other hand are sounds that contain useful information for the receiver. Thus, sound may be a signal to some and noise to others (ACA IMM, 2006).

Sound, and especially anthropogenic sound can induce a range of adverse effects in marine mammals. The effects can be insignificant or significant and direct or indirect. Important parameters for the evaluation of effects of underwater sound are the received peak pressure (received level, RL), the acoustic energy (sound exposure level, SEL), the duration of the signal and its repetition rate, its spectral type, frequency range and spectral energy distribution, directionality and signal rise time.

The defining parameters for acoustic effects are those of the received signal (immission) rather than the emitted signal (emission) and the propagation processes. Those gain importance only if the received levels cannot be measured directly and have to be modelled. Ultimately, it is the receiver’s sensitivity for the particular signal parameter which defines the acoustic effect.

Acoustic effects in marine mammals can be divided into physiological responses such as stress, acoustic masking, behavioral reactions and physiological or physical impairment. In extreme cases, acoustic effects can result in the death of the ensonified individual. Most of these processes are still poorly understood in marine mammals. The auditory studies presented in this dissertation focussed on the following aspects:

- Individual hearing sensitivity; only few animals in each of the two marine mammals species of interest have been tested for their hearing sensitivity. The psychophysical method, which was predominantly used in previous hearing studies, requires the trained locomotor response of the test subjects upon the perception of the test stimulus. The AEP method allows more rapid auditory testing without any active participation of wild animals.
- Acoustic masking; anthropogenic sound may impede the perception of meaningful acoustic signals by a marine mammal (NRC, 2003; Janik, 2005). The amount of masking depends on the acoustic characteristics of the received signal and the auditory capabilities of the receivers’ auditory system.
- Temporary threshold shift (TTS) in auditory sensitivity; the exposure to intense sounds may cause metabolic exhaustion or mechanical distortion of the sensory cells (Au and Hastings, 2008). As long as the sensory cells (hair cells) are not permanently damaged and eventually die, this effect should be reversible within 30 days, otherwise it is considered to be a permanent threshold shift (PTS).

A more detailed discussion of these aspects and effects is provided in the particular chapters.

As in most mammalian species the hearing system in marine mammals is the most sensitive organ with regard to pressure changes. Numerous tests with terrestrial animals reveal, for example, that hearing is damaged at lower sound intensities than other organ systems (Office of the Surgeon General, 1991). The effect most dealt with is hearing loss.

The complete passive and active acoustic complex of many marine mammal species underwent different evolutionary adaptations compared to their terrestrial relatives (Wartzok and Ketten, 1999) including a shift of the frequencies used for communication toward infrasonic as well as ultrasonic frequencies combined with an increased acoustic sensitivity over broad frequency bandwidths. Accordingly, the assessment of the possible impact of noise on marine mammals has mainly to deal with the possible impairment or damage of the auditory apparatus of the animals since, in contrast to the other organs, the lowest threshold regarding impairment or damage is presumably to be found there. Nevertheless, intense sound can also cause non-auditory effects such as rupture and hemorrhages in the air-filled compartments of the lungs and intestines (Goertner, 1982).

EDITORIAL NOTES

The dissertation consists of five chapters, each representing separate studies on auditory aspects. The study presented in chapter 3 was already published, the one presented in chapter 4 is conditionally accepted for publication, both in peer-reviewed journals. The remaining chapters were written in a format suitable for submission to peer-reviewed scientific journals.

All chapters are written in U.S.-English. This style was chosen to allow immediate submission of the remaining chapters to a U.S.-based journal. The text of the published manuscript was adapted to this style (with permission of the editor). Changes compared to the published version are apparent with regard to spelling, grammar, position of the figures and the format of the literature citations. The wording, however, was kept identical to the published version (except for one erratum – see footnote in chapter 3).

The references cited in each study are listed in each chapter separately while references for the introduction and synthesis are listed together at the end of the dissertation. The individual referencing leads to a duplication of some references throughout this dissertation.

DEVELOPMENT AND TESTING OF A RAPID METHOD FOR AUDITORY
SCREENING IN HARBOR PORPOISES (*PHOCOENA PHOCOENA*)

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ABSTRACT

Every year numerous harbor porpoises (*Phocoena phocoena*) are found alive along the Dutch shoreline and brought to a rehabilitation facility in the Netherlands. Many of these rescued animals can be successfully rehabilitated and eventually released after a period under intense medical care. However, the cause of their initial stranding often remains unclear. A compromised hearing sensitivity is among the likely causes of stranding as it would impede the animals' ability to echolocate and consequently avoid the proximity of nearshore areas. Irrespective of its cause, impaired hearing can have a strong negative impact on an animal's foraging abilities. With regard to the decision on the release after rehabilitation, knowledge of the animal's auditory status is therefore important and would improve the survival of released animals. Therefore, an auditory test method was established as a standard procedure of the medical screening prior to the release. The procedure is based on the auditory-evoked potential method and was successfully applied to six porpoises. The resulting audiograms are comparable with previous data obtained with the AEP and behavioral test. They showed individual variability, but none of the animals was diagnosed deaf or severely hearing-impaired.

INTRODUCTION

Harbor porpoises (*Phocoena phocoena*) are the only toothed whale species abundant in the North Sea waters of the Netherlands. Every year numerous animals are found alive along the Dutch shoreline and brought to the Dolphin Research and Rescue Centre, Harderwijk, the Netherlands. The cause of their stranding often remains unclear. Several factors such as pollution, habitat depletion and reduced food availability may cause a reduced immune system, leading to bacterial infections and finally to the stranding of weakened or sick animals. Another important factor causing an animal to strand could be anthropogenic sound, which can, besides other effects, lead to behavioral reactions or to an impairment of the auditory sensitivity in marine mammals. Compromised hearing sensitivity would impede the animal's ability to echolocate. It would thereby diminish the animal's potential to detect situations where it enters the closest vicinity of nearshore areas where the risk of stranding increases, e.g. in the surf zone or the extensive tidal flats of the Wadden Sea. Irrespective of whether an acoustic disability was caused by anthropogenic sounds, infections or genetically conditioned, it would also have a strong impact on an animal's foraging abilities and thus reduce its chance of survival in the wild.

Many of these rescued animals can be successfully rehabilitated and eventually released after a period under intense medical care. So far, however, the medical tests conducted within the screening procedure prior to release did not include testing the auditory status of the animals. Knowledge of the animals' auditory health was considered important for decisions on rehabilitation and release and would certainly improve the survival rate of released animals.

A dolphin found to be deaf and mute (Ridgway and Carder, 1997) may survive in the wild benefiting from cooperative behavior within its social group or being able to chase fish in waters with good visibility. For solitarily living animals like the harbor porpoise, which inhabits the murky waters of the North Sea, this is not an option.

The aim of this study was to establish a diagnostic tool to rapidly screen the hearing sensitivity of harbor porpoises without behavioral training but tolerating to be handled by humans. The resulting information would allow judging the overall health status of animals prior to their release on a more robust basis. In addition, such data would add to the available data set on auditory sensitivity and individual variation in harbor porpoises. So far, there is a limited data set with two complete audiograms measured with behavioral techniques (Andersen, 1970; Kastelein *et al.*, 2002) and partial audiograms and related auditory parameters measured by means of auditory-evoked potential (AEP) measurements (Popov *et al.*, 2006; Lucke *et al.*, 2007).

The classical method to test hearing in marine mammals is based on behavioral training of the animal and its locomotor response upon perception of a sound stimulus. The measurement of AEPs in contrast allows passive measurement of the auditory perception of a stimulus without active participation of the tested animal. This method is adopted from human audiometry for hearing tests in infants and other patients unwilling or unable to participate in the tests. It is a completely benign method. Surface electrodes are attached to

the skin and all measurements occur passively. The only stress to the animals is the attachment of the AEP electrodes (attached to the skin via suction cups) and the exposure to test signals.

The animal must remain as motionless as possible while floating near the surface with only the blowhole above the water. Procedures have already been adopted for stranded animals during the initial phase of their rehabilitation in Harderwijk because the animals are often too weak to swim and come to the surface to breath. These animals are either kept at the surface by a person in the water or by holding the animals in a floating stretcher.

The AEP method for auditory tests was successfully used on harbor porpoises in studies on different toothed whale species (Nachtigall *et al.*, 2003, 2004; Finneran *et al.*, 2002, 2005). However, in most studies the animals were trained to actively participate in the hearing studies. This reduced available time to measure the AEP to several minutes per day (e.g. Lucke *et al.*, 2007). Finneran and Houser (2007) showed that it is possible to prolong this period for AEP measurements by using hydrophones embedded into suction cups (jawphones) and keeping the animal in a stretcher at the water surface. By using these jawphones it is possible to position the sound source in a fixed position at the head of the animal, thereby ensuring that the stimuli to the animals are always delivered at the preselected sound intensity. This technique was previously used in several auditory studies on cetaceans (Moore *et al.*, 1995; Brill *et al.*, 2001; Finneran and Houser, 2007) and shown to provide reliable data.

MATERIAL AND METHODS

A. SUBJECT AND FACILITY

Auditory measurements were conducted on six stranded harbor porpoises (table 1) held either for their rehabilitation at Netherlands Cetacean Research and Rehabilitation Centre (SOS Dolfijn) at the Dolfinarium Harderwijk, Harderwijk, the Netherlands, or permanently at the dolphinarium's harbor porpoise enclosure. The health status of all harbor porpoises was examined prior to the measurements. Any animals showing signs of reduced fitness were excluded from the study to prevent aggravation. All animals were unседated. The breathing rate of the animals was constantly monitored during the test periods. Upon an increase of the rate above normal levels as well as strong behavioral activity the tests were immediately stopped and the animal was brought back into the main pool.

Table 1. List of all harbor porpoises tested in this study. Sex, estimated age and dates of arrival at the rehabilitation centre and auditory testing are given along with an internal reference number.

Code	Sex	Age (estd.)	In rehabilitation since	Date of testing
PpSH122	Female	2	04.01.2007	05.05.2008
PpSH125	Male	2	11.02.2007	14./15.05.2007
PpSH127	Female	3	02.03.2007	14./15.05.2007
PpSH129	Male	3	24.03.2007	14./15.05.2007
PpSH133	Male	2	12.03.2008	06./07.05.2008
PpSH135	Female	2	21.03.2008	06./07.05.2008

To conduct the AEP measurements the animals were removed from their pool and placed on a custom stretcher suspended into a test tank. This tank was 250 cm long, 61 cm wide, 57 cm high and made of wood. During the auditory measurements it was filled with water up to a level just below the animals blow hole, thereby ensuring ease of breathing for the animal. The animal's back was covered with wet towels, which were constantly rinsed to avoid drying of the skin during the test period.

B. RESPONSE ACQUISITION

Three surface electrodes (active, reference and ground) were placed along the dorsal midline of the harbor porpoise. The active electrode was placed 7.5 cm behind the animal's blowhole (based on data from Lucke *et al.*, 2007), the reference electrode was attached slightly lateral below the dorsal fin, and the ground electrode halfway between the two. All electrodes were 10 mm AgCl-cup electrodes mounted into standard suction cups. The electrode responses were filtered (high pass cut-off: 300 Hz, low pass cut-off: 3 kHz) and amplified 10,000 times by a low noise differential biopotential amplifier (GRASS IP511) before being fed into a A/D conversion card (NI-DAQ 6062E) at a digitization rate of 40 kHz. A single data point consisted of the averaged response of 500 AEP responses to the identical type and intensity of stimulus. All AEP responses were recorded and analyzed in real-time using custom-made software (A. Ya. Supin, Russia).

C. STIMULUS TYPE

The same custom made software was also used to generate the AEP stimuli. All stimuli were sinusoidally amplitude-modulated (SAM) signals of 20.8 ms duration. The signals were fully modulated (modulation depth = 100 %) and their received sound pressure level (SPL) was kept constant during the 500 presentations required to measure one data point at the selected carrier frequency. This type of signal elicits a so-called envelope following response

(EFR) in the auditory system which has a fundamental frequency at the amplitude-modulation frequency used (Kuwada *et al.*, 1986; Dolphin and Mountain, 1993). This allows a more frequency-specific analysis of the hearing sensitivity than responses evoked by other types of acoustic stimuli. Several sequences of AEP-stimuli at different SPL's (with 500 presentations at each level) had to be tested to acquire the hearing threshold at one selected carrier frequency and to achieve enough data points for a threshold analysis. The step size between successive stimulus levels was varied according to the response amplitude achieved at the previous SPL. At supra-threshold levels (presentation levels clearly above threshold) the step size was increased (maximum: 5 dB), thereby reducing the total number of measurements required and time needed for the complete test sequence. At near threshold levels, on the other hand, the step size between SPLs of successive measurements was decreased (minimum: 1 dB), thus providing a higher resolution.

D. THRESHOLD ANALYSIS

The test procedure comprises measurements at various SPLs, including levels which result in no auditory evoked response. The evoked responses are analyzed in the frequency domain (by means of a Fast-Fourier transformation of the neuronal response). The amplitude of the resulting EFR is correlated to the perceived stimulus level. This allows determination of the hearing threshold at the selected carrier frequency by extrapolating from the measured EFR amplitudes to zero (Supin *et al.*, 2001). In this context, it is essential to decide whether or not an auditory evoked response is present or absent in a data set. Detection of EFRs near threshold levels is the most challenging aspect (Gans *et al.*, 1992; Dobie and Wilson, 1995). A variety of analysis techniques exist to determine presence or absence of responses (Finneran *et al.*, 2007). In this study two methods were applied, each providing a different degree of objectivity and leading to different procedures of defining a hearing threshold:

- 1.) The lowest detected response at the base frequency of the EFR response (i.e. the modulation frequency) was determined for each data set. The response amplitude at the modulation frequency (1.2 kHz) had to be higher than those at the adjacent ten frequencies (five below and five above, covering a frequency range 700 Hz) to constitute "EFR present."
- 2.) An F-test was applied to all data sets from two animals (PpSH 125 and PpSH 129). The noise power at the modulation frequency (1.2 kHz) was thereby compared to the noise power of the adjacent frequencies ($m = 10$; $F_{crit} = F(2, 20)$) (Dobie and Wilson, 1996).

An upper limit was set in both methods by only including data sets with EFR responses below 0.5 μV in the regression analysis. Each series of data sets also had to include measurements at SPLs where the EFR was decided to be absent.

E. BACKGROUND NOISE

Sound emissions from external noise sources at the facility were kept to a minimum during the measurements to reduce a potential masking effect. Transmission of external sound into the test tank via the ground was reduced by resting the tank on wood. Background noise was recorded over a frequency range from 1 Hz to 160 kHz using a custom-made software (Pavan *et al.*, 2001) via a hydrophone (Reson TC 4014) and an adjustable preamplifier (Etec B1501).

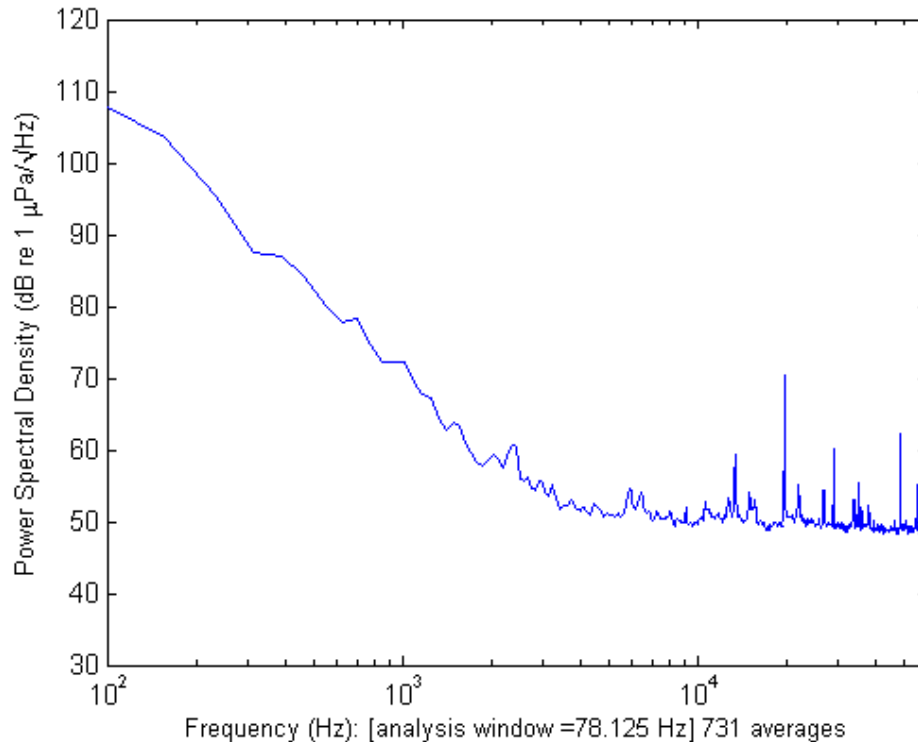


Figure 1. Background noise in the test tank was dominated by low frequency noise from external sound sources which decrease with increasing frequencies. At frequencies above approximately 5 kHz the background noise then reached levels below the self noise of the measurement system.

F. STIMULUS PRESENTATION

AEP stimuli were presented via two suction cup hydrophones (jawphones). These transducers consisted of two standard ball hydrophones (SRD HS 150) which were molded into two-component silicon rubber. A calibration of the jawphones (figure 2) was conducted by measuring the underwater rms sound pressure levels (SPLs) each at a distance of 15 cm from a reference hydrophone (Finneran and Houser, 2006).

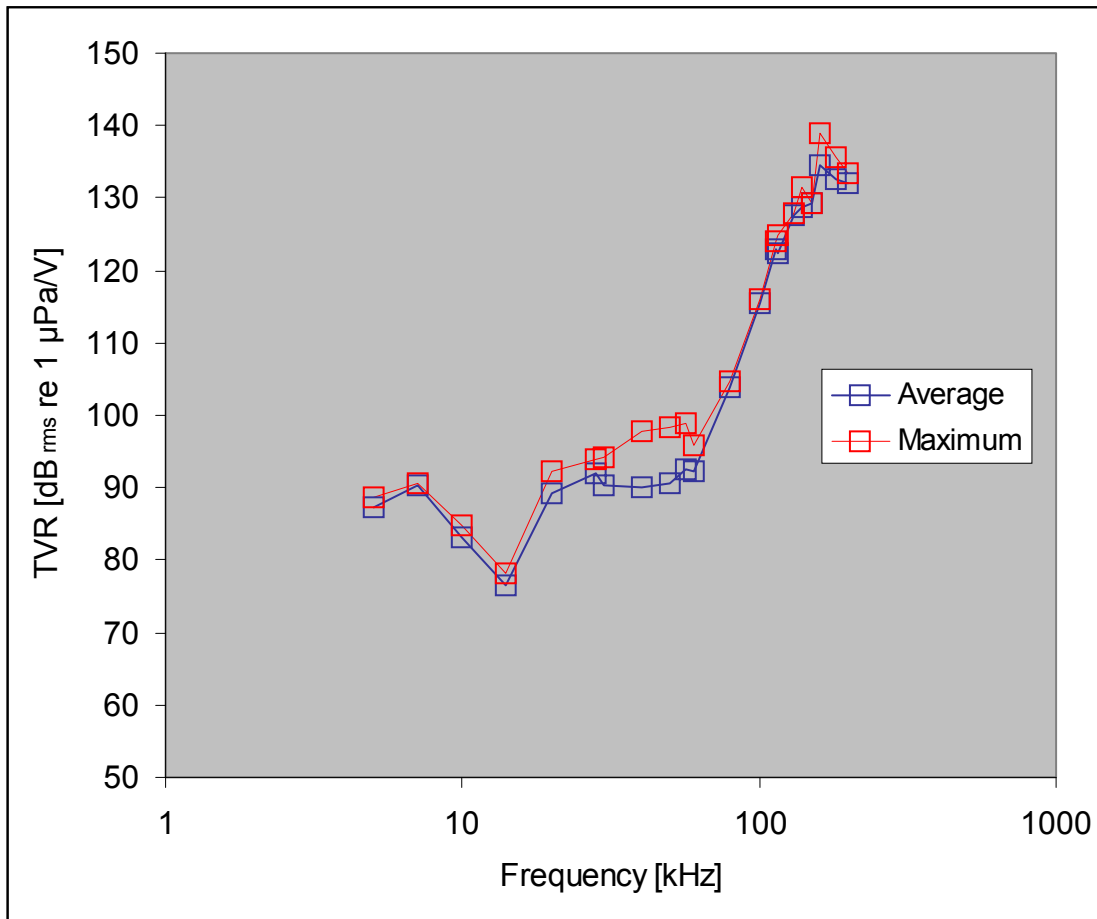


Figure 2. Transmitting voltage response (TVR) of the two jawphones used for stimulus presentation. The TVR is plotted as average for both hydrophones (blue symbols/line) and for the more sensitive hydrophone, i.e. the one transmitting the acoustic stimuli at higher SPLs (red symbols/line).

It was previously demonstrated in other toothed whale species that these animals have a high acoustic sensitivity along the lower jaw and especially in the pan bone region (Brill *et al.*, 1988; Møhl *et al.*, 1999; Finneran and Houser, 2006). This position and, additionally, the area around the opening of the outer ear canal were also found to be most sensitive to acoustic stimulation in harbor porpoises. Subsequently, the jawphones were placed in either of these positions depending on the animal's size. In larger animals, whose heads extended beyond the stretcher, suction cups were placed below the outer ear canal. In smaller animals, those cups were placed on the lower jaw.

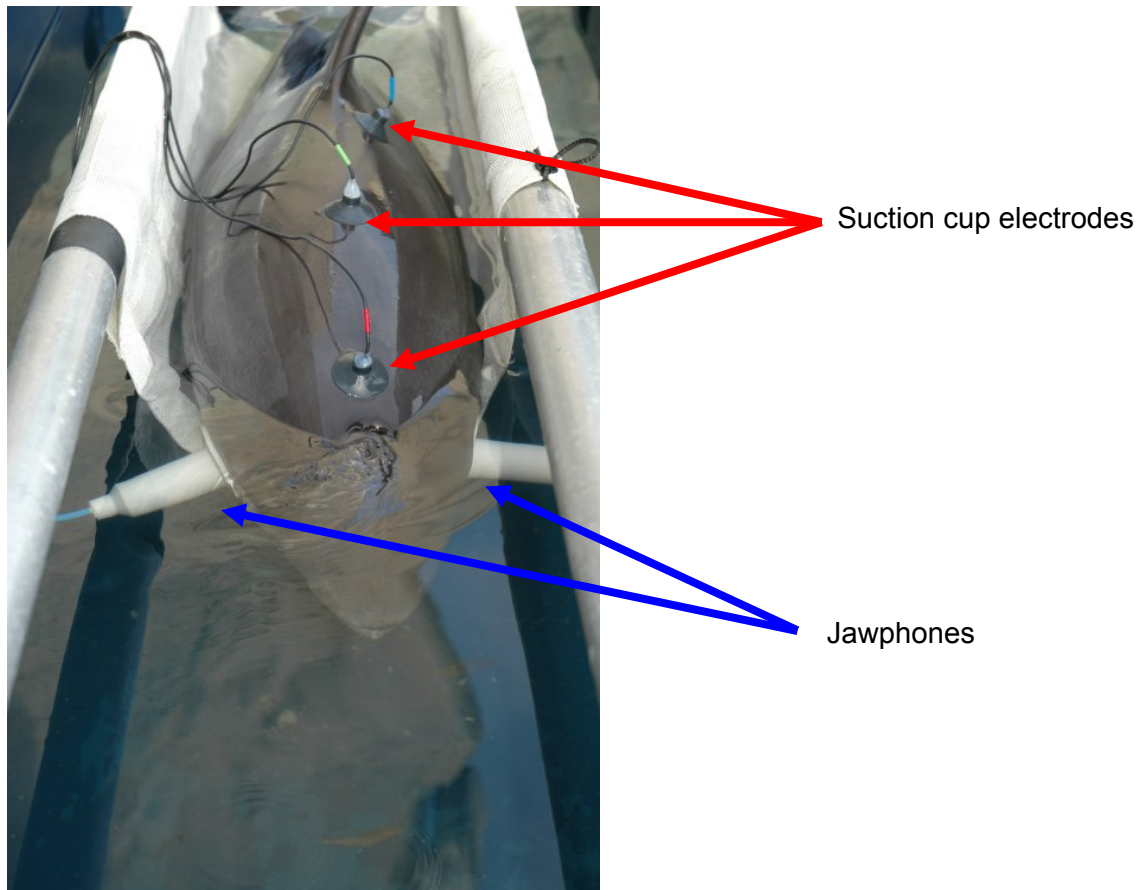


Figure 3. Harbor porpoise hanging in a stretcher in the test tank with jawphones (sound source) and suction cup electrodes attached.

G. MODULATION RATE TRANSFER FUNCTION

A modulation rate transfer function (MRTF) was determined in one animal (PpSH 135) to identify the best amplitude modulation rate of the sound stimuli. By systematically increasing the modulation rate of a 100 kHz carrier signal, the resulting EFR was found to be at its maximum at 1.2 kHz, which is consistent with results from previous experiments in harbor porpoises (Lucke *et al.*, 2007). Applying this modulation rate to the SAM stimuli at all frequencies should provide the best signal-to-noise ratio between the EFR and the non-acoustically induced neuronal potentials (figure 4) and thus increase the resolution for threshold analysis.

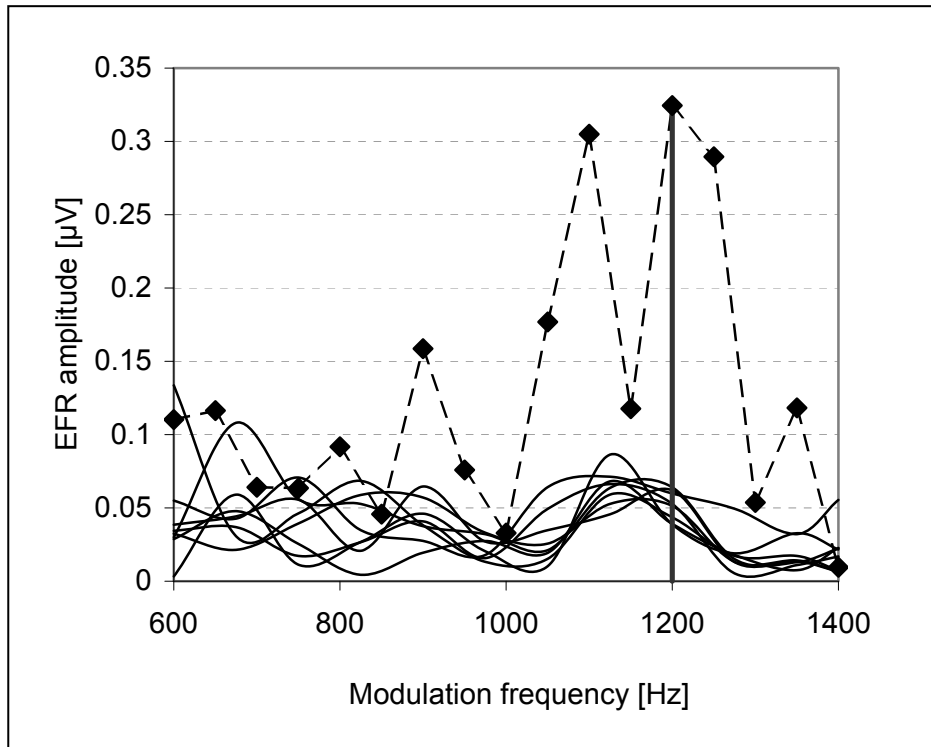


Figure 4. Modulation rate transfer function (dashed line) of a harbor porpoise (code PpSH135). Acoustic stimuli were presented via suction hydrophones attached to the head of harbor porpoises suspended in a stretcher at the water surface of a test tank. The envelope following responses (EFR, black diamonds) were evoked at different amplitude modulation rates of a 100 kHz carrier signal. Solid curves represent neuronal responses measured without acoustic stimulation, thus representing the neuronal “background noise.” The solid vertical line indicates the modulation frequency yielding the highest EFR.

III. RESULTS

A. BEHAVIOR

Three animals (PpSH 125, 127 and 135) were calm during the test period, not showing any obvious aversive reaction in response to the test procedure (i.e. placement in a stretcher and presentation of acoustic stimuli). Apart from breathing activity the only locomotor behavior observed in these three animals was a repeated movement of the head from one side to the other (probably connected with a echolocation scanning of their new environment). The other three harbor porpoises, in contrast, were found to be significantly more active throughout the test procedure. They either tried repeatedly to lift their head out of the water, lifted their fluke or arched their whole body in a horizontal or vertical plane. As long as these movements were comparatively slow and constant, they were considered not to be an indicator of increased stress and the test procedure was continued.

B. HEARING THRESHOLDS

Complete audiograms covering their full auditory range were recorded in the five animals, with a reduced set of thresholds for the active animals. The data measured in one of the active animals (PpSH 122) did not provide sufficient quality to determine more than two threshold values. The audiograms of the other two active animals were also reduced in the number of thresholds obtained but still covering a wider range of frequencies. All data are listed in table 2 and displayed in figures 5 and 6.

Table 2. Auditory threshold values measured in six harbor porpoises with an electrophysiological method (AEP method) at various frequencies. Acoustic stimuli were presented via suction hydrophones while the animals were suspended in a stretcher at the water surface of a test tank. The overall behavioral status was assessed over the entire test period. Empty cells represent frequencies which were not tested or did not reveal threshold values.

	PpSH 125	PpSH 127	PpSH 135	PpSH 129	PpSH 133	PpSH 122
Behavior	calm			active		
Frequency	Hearing threshold					
[kHz]	[dB _{rms} re 1 µPa]					
10	72.4	62.4	35.1	-	68.0	-
20	-	68.5	-	68.1	48.9	-
30	61.5	-	-	-	-	-
40	54.6	39.2	28.7	48.1	43.4	-
50	-	32.9	-	-		-
60	58.1	53.0	44.0	71.4	59.6	-
70	37.1	-	-	-		-
80	52.0	47.4	43.8	98.7	74.7	-
90	-	51.0	-	-	-	-
100	60.8	51.2	74.2	89.3	98.7	-
110	49.8	58.9	-	-		-
120	27.3	-	37.1	71.8	52.7	62.4
130	10.9	20.3	-	-	-	-
140	12.5	-	67.6	-	63.0	68.6
150	-	-	-	-	-	-
160	110.3	108.6	127.3	120.9	119.1	-
170	103.7	131.5	133.5	-	-	-
180	124.5	118.5	-	-		-

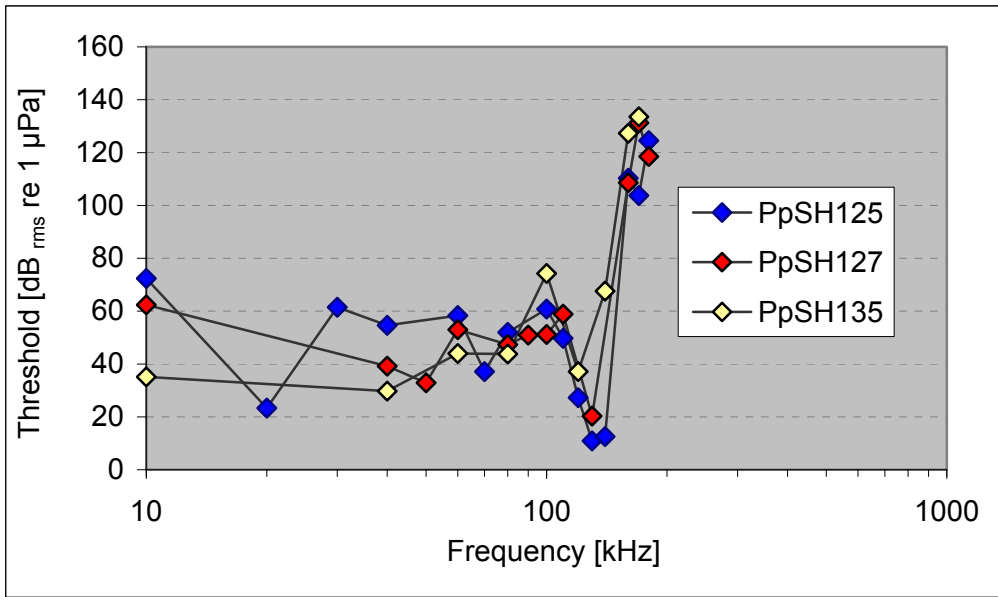


Figure 5. Auditory threshold values (colored diamonds) of three harbor porpoises showing no behavioral activity during the test procedure. Acoustic stimuli were presented via suction hydrophones attached to the head of the harbor porpoises while the animals were suspended in a stretcher at the water surface of a test tank. All measurements were conducted using the auditory evoked potential method.

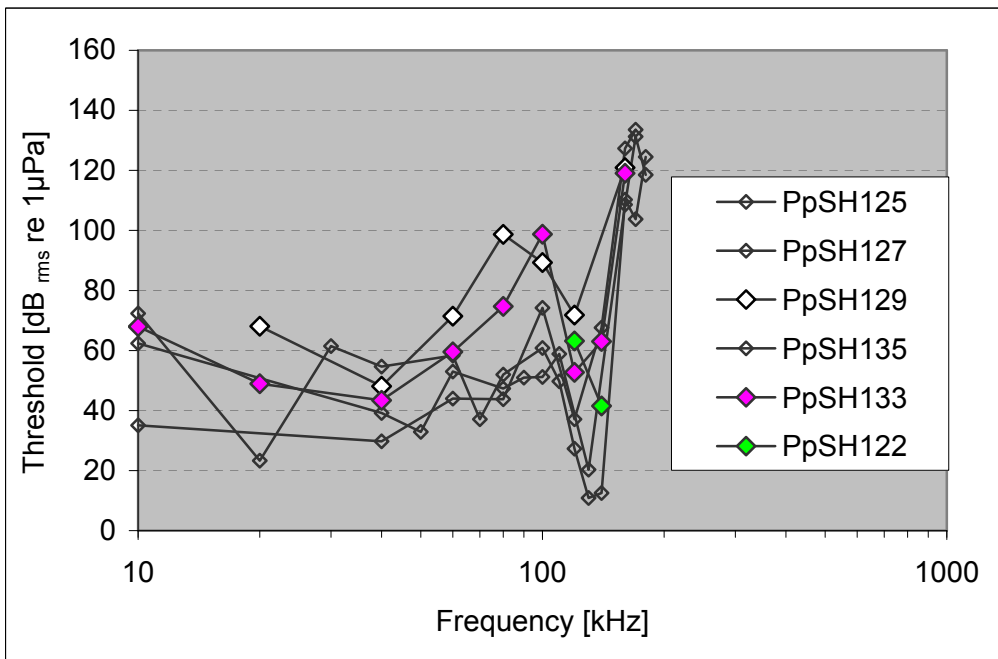


Figure 6. Auditory threshold values (colored diamonds) of three harbor porpoises behaviorally active during the test procedure. Acoustic stimuli were presented via suction hydrophones attached to the head of the harbor porpoises while the animals were suspended in a stretcher at the water surface of a test tank. All measurements were conducted using the auditory evoked potential method. Auditory threshold values of three harbor porpoises calm during the procedure are shown for comparison (open diamonds).

When averaged over all frequencies, the difference in auditory threshold between the three calm and three active animals was 20.3 dB. The largest threshold elevation (maximum: 39 dB) in the active animals was found at 20 kHz and at 80 -120 kHz while thresholds were at comparable levels at 40 kHz and 160 kHz (with a difference of 4.6 dB).

A comparison of auditory threshold values measured in the three calm animals with results of two auditory studies on harbor porpoises using behavioral test procedures (Andersen, 1970; Kastelein *et al.*, 2002) is shown in figure 7.

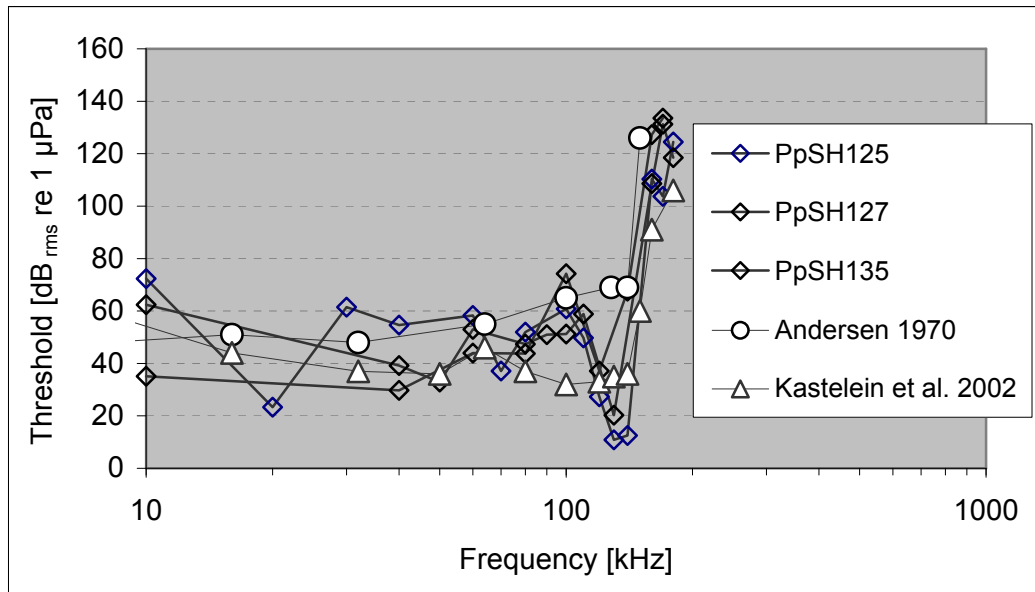


Figure 7. Comparison of auditory threshold values of three harbor porpoises tested with an electrophysiological method (AEP-method) with auditory threshold values of two harbor porpoises measured with a behavioral test procedure (Andersen, 1970; Kastelein *et al.*, 2002).

The application of the two analysis methods to the measured EFR data results in different hearing thresholds. Figures 8 and 9 show the comparative results for one of the active and one of the calm animals.

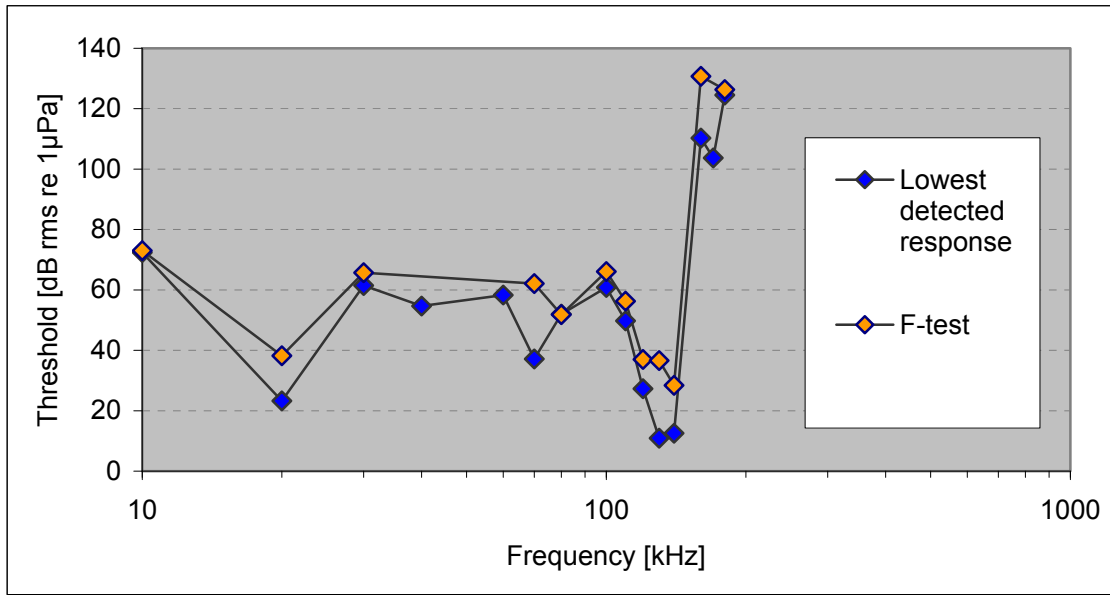


Figure 8. Comparison of auditory threshold values of harbor porpoise PpSH 125 determined with two analysis methods. Orange diamonds represent threshold values obtained by applying an F-test ($F_{2, 20}$) to all data sets, orange diamonds resulted from determining the lowest detectable response.

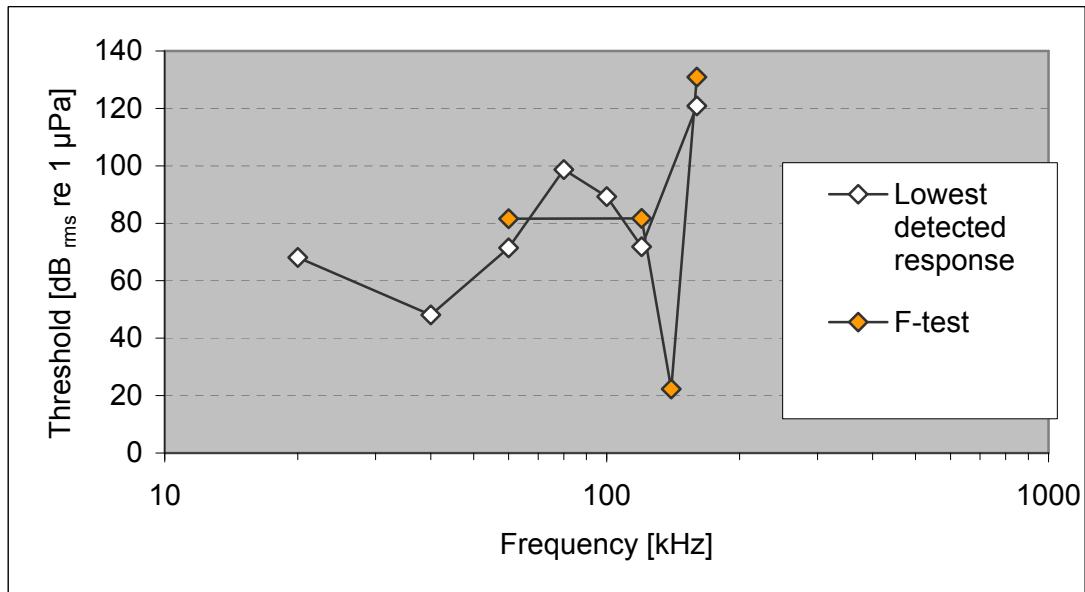


Figure 9. Comparison of auditory threshold values of harbor porpoise PpSH 129 determined with two analysis methods. Orange diamonds represent threshold values obtained by applying an F-test to all data sets, white diamonds resulted from determining the lowest detectable response.

The F-test leads to systematically elevated hearing thresholds as compared to the method of the lowest detectable response. Furthermore, the number of threshold values that can be determined based on the data sets is increased by using the latter method.

DISCUSSION

The methodological approach used in this study was shown to result in hearing threshold data of high diagnostic value. With the described system and method, the auditory measurements provide sufficient information to assess the auditory status of harbor porpoises in rehabilitation in general and specifically prior to their possible release. These measurements can be conducted within a limited time period even in untrained harbor porpoises.

Given the individual variability in hearing sensitivity the threshold levels in the three calm harbor porpoises showed a good match. The audiograms of these animals had the same W-shaped curve as found by Andersen (1970) and Kastelein *et al.* (2002) and also compared well to the levels determined in these two prior behavioral studies. Acoustic masking from background noise can be ruled out at the frequencies tested. Hearing thresholds measured in the calm animals PpSH 125, PpSH 127 and PpSH 135 can thus be regarded as absolute hearing thresholds. Auditory studies on marine mammals comparing results from both techniques in one animal previously showed some systematic differences in threshold levels, especially at the low and high ends of the frequency range (Yuen *et al.*, 2005; Finneran and Houser, 2006) – with behavioral testing revealing slightly lower thresholds. A comparable study has not yet been conducted in harbor porpoises. Because it is impossible to conduct a comparative auditory study in untrained harbor porpoises, it will remain unclear whether or not such a difference between neurophysiologic and behavioral results exists in harbor porpoises or whether such differences are individual, species-specific or attributable to methodological aspects.

The threshold levels determined for the active animals, on the other hand, were clearly elevated. Inevitably, the level of non-acoustic neuronal potentials was elevated in the restrained, yet active animals as limited movement during the procedure was permitted. It is evident that the near threshold EFRs were masked by this increased level of neuronal (myogenic) potentials. The amplitude of the EFRs is not in linear correlation with the SPL of the received acoustic stimuli, and EFR values usually show a slower decrease at near threshold levels. Such masking of the lowest values results in higher hearing thresholds, irrespective of the method used to determine the thresholds (extrapolation vs. level of the last detectable significant EFR value). The hearing thresholds of the harbor porpoises PpSH 129, PpSH 133 and PpSH 122 must be regarded as masked hearing thresholds. Nevertheless, the rapid auditory screening of harbor porpoises remains a valid and useful approach even in active animals. Even masked thresholds allow conclusions regarding the overall hearing capability of the tested animal. If the activity status of the animal is taken into account in the diagnostic assessment, the absolute thresholds may be extrapolated from the masked thresholds with some degree of confidence.

The statistical analysis (F-test) was too conservative to yield useful results. It reduced the general quality of the results by reducing the number of data points that could be used to determine individual threshold levels and the amount of threshold values determined in total. It also led to elevated threshold levels. The other method, the determination of the lowest

detectable response, resulted in a higher number of data points for the determination of each threshold, an increased number of frequencies for which a threshold can be determined and threshold levels comparable to those established behaviorally. By applying strict rules and limitations to this type of analysis, this method also avoids observer bias and can constitute an objective response detection technique. Its advantage compared to the F-test remains independent of the signal-to-noise ratio of the neuronal responses which was influenced by the activity status of the harbor porpoises in this study.

By calculating the received SPLs based on the transmitting response of the more sensitive hydrophone (i.e. the one transmitting the acoustic stimuli at higher SPLs), the resulting hearing thresholds may be underestimated. This more conservative approach was chosen as a safety measure with regard to the diagnostic value of the data for the decision on a potential release of the animals. This approach implies that the auditory system must be capable of perceiving an acoustic stimulus under real-life conditions at its faintest levels even when it is a directional cue coming from one side rather than levelling out both sides. Differences in received levels between both sides would give the animal directional cues rather than defining its threshold.

Another criticism with regard to bilateral stimulus presentation could be that a slight difference in placement of the two jawphones relative to each other would cause phase differences in the auditory processing. As a consequence the perception could be impeded, an effect that could be avoided by a single-sided stimulus presentation. The strong difference in the resulting threshold levels between single-sided and both-sided presentation of the acoustic stimuli contradicts this assumption as the both-sided presentation clearly enhances the presentation. As other odontocetes, harbor porpoises are likely to receive acoustic input over a wider area stretching along their lower jaws and an area around their (mainly dysfunctional) outer ear canals. Under real-life conditions the animal's hearing system would have to integrate the acoustic input over the whole area without any neuronal interferences. Still, the placement of the jawphones was conducted as consistently as possible. However, two different positions had to be used due to the size of the animals compared to the dimensions of the stretcher. Further variations in the placement of the jawphones were unavoidable due to movements of the animals during the test procedure, which repeatedly resulted in shifting of the suction cups. Those shifts were comparatively small and occurred over a period of several minutes. A difference in auditory sensitivity at different positions would result in variations in the evoked neuronal responses' relative height, thus only influencing the resolution of the threshold analysis. Its effects on the absolute height of the response amplitude, in contrast, should be negligible within the measurement procedure for a single data point.

The hearing threshold could be measured at even lower frequencies than in this study (i.e. below 10 kHz). However, a lower limit is set in principle by the amplitude modulation rate and should ideally be at least a multiple of it (Lucke *et al.*, 2007). 10 kHz represents a sensible lower frequency limit in this context as the auditory screening is mainly focused on the animals' capability to perceive echoes of its own acoustic ultrasound signals while

echolocating. The highest frequencies tested fell within the functional range of the recording system, thus avoiding aliasing effects.

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IN-AIR HEARING SENSITIVITY IN HARBOR SEALS (*PHOCA VITULINA*)
FROM THE NORTH SEA AS A BASELINE FOR LONG-TERM AUDITORY
MONITORING

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ABSTRACT

The planned construction of a large number of offshore wind turbines (OWTs) in the German Bight raised concern because of the potential auditory impact of this activity on the harbor seal (*Phoca vitulina*). Due to the comparatively high level of anthropogenic activities in the southern North Sea it is likely that the ambient noise level in these waters is raised already prior to the installation of the OWTs compared to other areas. It was therefore hypothesized that adult harbor seals from the German Bight in general may already show a reduced hearing sensitivity compared to individuals from other areas. In contrast, juvenile and sub-adult individuals from the North Sea have been exposed to no significant levels or lesser amounts of noise. Consequently, these animals should have an undisturbed hearing sensitivity or show a lower grade of hearing impairment compared to adult animals. Individuals from these age groups were chosen for auditory measurements to acquire reference data for the southern North Sea sub-population of harbor seals. This information will serve as the baseline for monitoring long-term development of the acoustic health of these animals.

Six harbor seal pups and four sub-adult harbor seals were tested for their in-air hearing sensitivity. The AEP method was used to conduct the auditory measurements while the animals were immobilized. Their hearing sensitivity was measured at frequencies between 0.7 kHz and 22.4 kHz short tone pips presented via headphones.

The resulting thresholds measured in all animals are in good agreement with previously published hearing data from other studies on harbor seals from the North Pacific and North Atlantic. This indicates that the chosen approach is suitable for further auditory monitoring of this sub-population. Moreover, the gathered data define a level of a healthy hearing system in harbor seals and can be used as a baseline for the assessment of auditory effects of an acute or chronic increase in their acoustic environment due to anthropogenic activities.

INTRODUCTION

Harbor seals (*Phoca vitulina*) are abundant along the northern European coastlines, thus inhabiting areas of increased anthropogenic use (OSPAR Commission, 2000). With their amphibious lifestyle they are confronted with human activities both above and under water. Recently, plans to build a large number of offshore wind turbines (OWTs) in the North Sea have been developed in all littoral countries. Based on information about auditory effects of intense sounds on marine mammals (Finneran *et al.*, 2002; Nachtigall *et al.*, 2003, 2004; NRC, 2005; Kastak *et al.*, 2007), concern was raised about the potential effects of the intense acoustic emissions on the harbor seals during the construction and operation of OWTs.

Even though the tactile sense is the most important sensory modality for harbor seals (to forage and navigate based on hydrodynamic cues; Dehnhardt *et al.*, 2001; Schusterman *et al.*, 2004), these animals also evolved an acute sense of hearing for in-air and underwater sounds. A number of behavioral psychophysical hearing tests were conducted on captive harbor seals over the past decades, both in air and underwater (Møhl, 1968; Terhune, 1988, 1991; Kastak and Schusterman, 1998; Kastak *et al.*, 1999; Holt *et al.*, 2001; Kastak *et al.*, 2005b). With an increasing number of auditory studies using auditory evoked potentials (AEPs) to measure the hearing sensitivity in marine mammals, this technique was recently also applied to captive harbor seals (Wolski *et al.*, 2003). The studies revealed that harbor seals have good auditory sensitivity in air – with a functional hearing range from 0.1 kHz to 30 kHz – (figure 1) and even wider for underwater sounds as they are able to perceive sounds from below 0.1 kHz to at least 128 kHz.

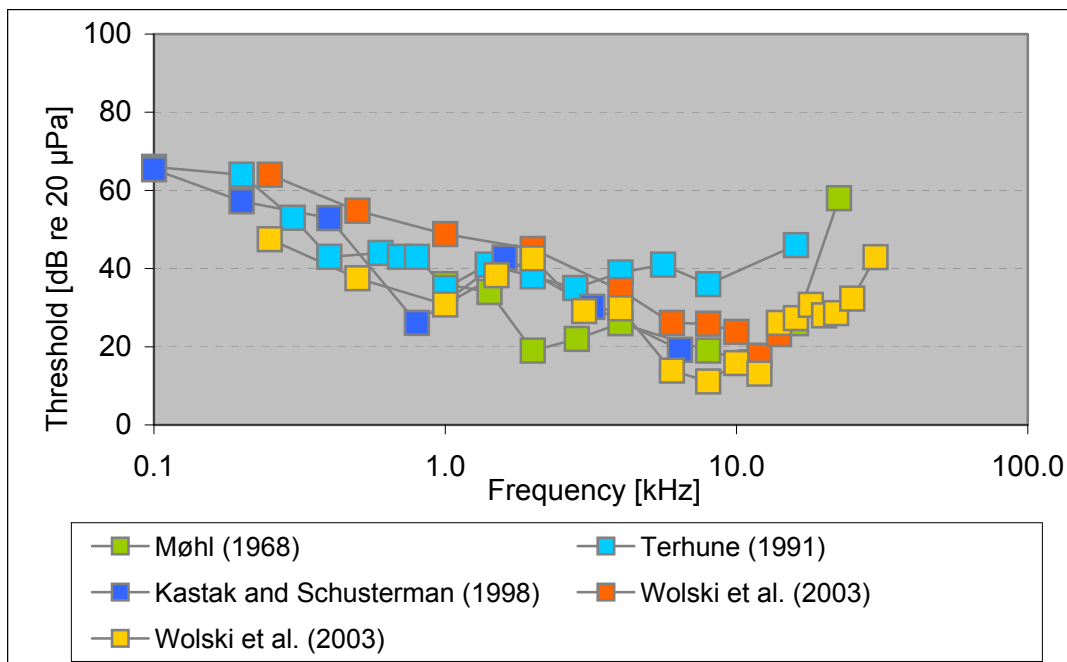


Figure 1. In-air hearing threshold data of adult harbor seals acquired in audiometric studies using psychophysical methods and different acoustic stimuli.

Like other phocid species, harbor seals use acoustic signals to interact with their environment. This involves vocalization (active) and listening (passive) for communication

purposes, both in air and underwater. In air, they rarely produce sounds (exceptions: pups – occasional guttural threats). In contrast to their behavior on land, they developed complex underwater vocal repertoires. Recent studies have shown that males are vocalizing underwater during mating season (Van Parijs *et al.*, 1999). This kind of vocalisation may be used to attract females but also in the context of territorial and competitive behavior with other males (Bjørgesæter *et al.*, 2004; Hanggi and Schusterman, 1994; van Parijs *et al.*, 1999, 2000a, 2000b). The known frequency range of the underwater roar of harbor seals is 0.4 to 4.3 kHz, with dominant frequencies between 0.4 to 0.8 kHz (Hanggi and Schusterman, 1994; Richardson *et al.*, 1995). Underwater sounds produced during mating season are often preceded by fore-flipper slaps on the water surface, which cause short click-like sounds (Richardson *et al.*, 1995). These slaps are 2 ms long and estimated to reach source levels of 186-199 dB_{pk-pk} re 1 µPa (Wahlberg *et al.*, 2002). The seals may also perform this behavior to show aggression. It remains unclear to which extent female harbor seals use sound actively, e.g. for mother-infant or individual recognition.

Seals may not be vitally dependent on their auditory system to survive as adult individuals, but a functional hearing system and the unrestricted perception of biologically meaningful sounds is a key issue for seal pups during the nursing phase whenever they are separated from their mothers. Reproductive success may be affected by sound-induced disturbance and thus would have implications on a population level. Beside these behavioral disruptions, acoustic signals may lead to short- or long-term displacement from valuable feeding or resting habitats and interrupt or prevent successful congregation of mating pairs. In extreme situations, intense sound may lead to injury in seals. At all levels of perceived intensity, noise is likely to cause stress in the animals, with unknown short- and long-term effects.

Consequently, important aspects in need of assessment in conjunction with the construction of wind farms in or near harbor seal habitats are the audibility range of wind turbine-related sounds for these animals and the potential effects of these sounds on their communication. The available auditory data provide a valuable baseline, but they have been collected in animals originating from other marine areas than the North Sea. As the amount of anthropogenic use strongly varies regionally, and the North Sea is among the most heavily used marine areas (OSPAR Commission, 2000), the seals tested so far may have had a different history of long-term noise exposure than those from the North Sea, and as a consequence a different hearing sensitivity. Furthermore, it can be hypothesized that adult harbor seals from the German Bight in general may already show a reduced hearing sensitivity compared to individuals from other areas (e.g. Norwegian waters). In contrast, juvenile and sub-adult individuals from the North Sea have been exposed to no significant or lesser amounts of noise. Consequently, these animals should have an undisturbed hearing sensitivity or show a lower grade of hearing impairment than adult animals. Individuals from these age groups were therefore chosen for auditory measurements to acquire reference data for the southern North Sea sub-population of harbor seals. This information will serve as the baseline for monitoring long-term development of the acoustic health of these animals.

MATERIAL AND METHODS

SUBJECTS AND FACILITY

The studies were conducted at the Seal Centre Friedrichskoog, Germany, where numerous (up to >70) wild seal pups are temporarily held under human care and successfully rehabilitated every year. All pups were initially found abandoned by the mothers for unclear reasons and brought to the Seal Centre. The tested animals were less than 4.5 months old and weighed less than 33 kg at the time of the experiments. These measurements were conducted in the Seal Centre's medical laboratory. During the measurements background noise was kept to a minimum to reduce acoustic masking.

Complementary tests were conducted on an adult seal (22-years-old female) permanently under human care in the Seal Centre. Access to wild seals was usually possible during routine seal catches in German and Danish Wadden Sea areas conducted twice a year as part of a health monitoring program. These catches take place on sandbanks where seals can be tested for up to 1.5 hours during low tide. All tests were conducted under continuous veterinary control (i.e. monitoring of respiration, heart rate, oxygen- and CO₂-concentration in all animals and blood tests prior to anesthesia in animals in rehabilitation).

STUDY DESIGN

To successfully conduct the planned measurements the study design had to take several aspects into consideration:

A measurement technique was required allowing comparable auditory data on wild and temporarily captive animals. The time constraints during the seal catches on the sandbanks made measurement of auditory evoked potentials (AEPs) appear the most suitable method. This rapid method is based on neuronal signals being elicited within the hearing system upon perception of a sound. These neuronal signals form an electric field in the body and can be measured as potentials even on the surface of the subjects' skin. To reliably detect these relatively small potentials within the multitude of non-auditory neuronal signals, the acoustic stimuli are repeatedly presented to the subject and the measured potentials are averaged coherently. All non-acoustic or incoherent neuronal potentials are reduced or completely eliminated. The best placement of the electrodes and the optimum acoustic stimuli to elicit AEPs were determined prior to the hearing threshold measurements on a trained harbor seal at the Seal Centre.

Auditory tests on wild seals would most likely elicit strong aversive reactions of the subjects once caught and restrained. The resulting myogenic neuronal signals would interfere with the auditory evoked neuronal signals (Au and Hastings, 2008) and impair the determination of the hearing thresholds. After different techniques to calm down or restrain the wild animals during the auditory measurements on the sandbanks had been tested without success, it was decided to immobilize the animals for the duration of the

measurements using diazepam (dosage: 0.02 ml/kg) and ketamine (dosage: 1.5-2 mg/kg) for dissociative sedation. The same approach was used for the measurements on the seal pups which were conducted indoors at the Seal Centre. All seals were continuously observed by an experienced veterinarian during the experiments and for at least one hour afterwards until they were found to behave normally again.

HARDWARE SETUP

A workstation (Tucker-Davis Technologies, TDT; System 3) was used to generate the AEP stimuli. Acoustic masking (i.e. the impaired perception of a sound due to the presence of another sound) of the AEP stimuli can best be avoided by reducing the surrounding sound (as in an anechoic chamber) or by dampening the environmental noise through the use of headphones. In this study the AEP stimuli were presented to the seals via headphones (Beyerdynamic DT 48 A) which provided 12 dB attenuation of the ambient noise. The evoked potentials were measured with needle electrodes (NIHON-Kohden) and fed into an input module (TDT RA 4 LI) and a bio-potential amplifier (TDT RA 4 PA), and subsequently into the TDT workstation for real-time display, further analysis and data storage. The recorded potentials were bandpass-filtered between 500 Hz and 2.5 kHz. Under normal measurement conditions 500 AEPs were averaged to achieve one data point.

Short tone pips consisting of 8-cycles cosine-gated sine waves (i.e. a bandwidth of 0.2 octaves; Reichmuth *et al.*, 2007) were used as AEP stimuli for frequencies at 1 kHz and above. The duration of these stimuli varied between 0,4 ms and 8 ms. To evoke AEPs at sufficient levels for lower frequencies, i.e. at 1.4 kHz and below the number of cycles was reduced to 3 (bandwidth \sim 0.8 octaves), thus providing an overlap in the frequency range tested with the two types of stimuli. As the frequency specificity of the signals and the amplitude of the evoked neuronal responses are negatively correlated, the best combination of the two factors had to be determined. The loss of frequency specificity was regarded as an acceptable trade-off for successful threshold measurement at the low frequencies as these were of special interest in the context of the windmill-related sounds.

Each data set was analyzed by measuring the peak-to-peak amplitude of the most prominent neuronal potential (in seals: potential V, see figure 2). An upper limit of 5 μ V (peak-to-peak) was chosen for the amplitude to include only measurements at comparable received sound pressure levels in the analysis. The data were tested with an F-test with $F_{(2,20)}$ degrees of freedom and a level of significance α of 0.05 to identify the presence of a statistically significant neuronal response near threshold levels. Moreover, only those data sets yielding a clearly identifiable peak of the neuronal wave V were included in the analysis. The hearing threshold was determined for each frequency by determining the zero-crossing of a linear regression function of the significant data points.

A low background noise level was maintained during all auditory measurements at the Seal Centre. In contrast, the hearing sensitivity of wild harbor seals was tested under varying environmental conditions. While most measurements were conducted under favorable

weather conditions (figure 3), wind speeds could vary and some AEP measurements had to be carried out at one location at moderate to high wind speeds (up to Bft. 6). Due to physical shelter from the wind at this measurement, the background noise is elevated only by up to 10 dB at lower frequencies (frequency range up to ~10 kHz) as compared to the other measurements.

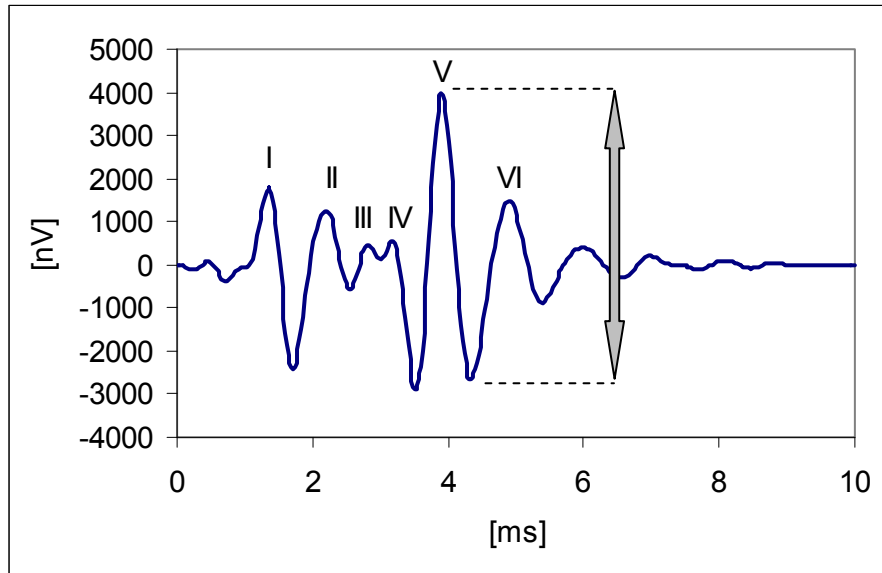


Figure 2. Auditory evoked potentials measured in a wild harbor seal (Pv 3327) after repeated exposure to short tone pips presented via headphones. A total of 1500 AEP potentials was averaged to determine this curve in an immobilized animal. The positive peaks of the AEP waves are numbered according to their succession (I-VI) (see Jewett and Williston, 1971). The arrow between the two dashed lines indicates the amplitude of wave V, which was analyzed as a response criterion in this study.

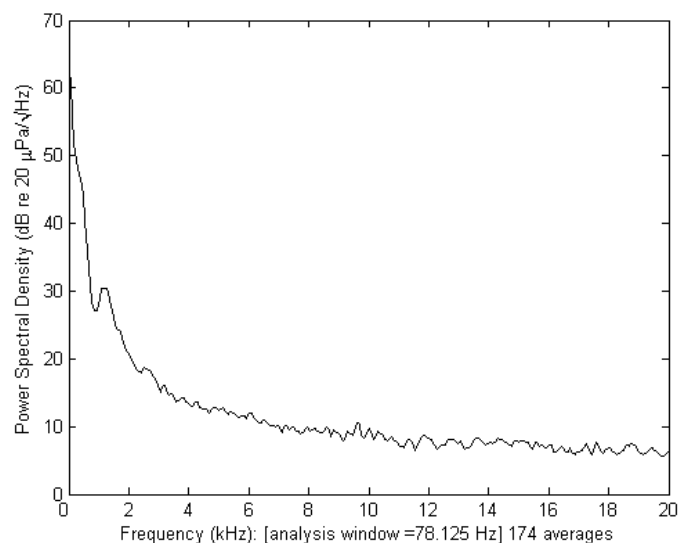


Figure 3. Background noise level (plotted as power spectral density) recorded in air on a sandbank under favourable acoustic conditions (very low level of wind-generated noise).

Background noise measurements were conducted for all measurement conditions, i.e. on the sandbanks and in the Seal Centre, to assess the potential acoustic masking of the perception of the AEP-stimuli. Recordings were made via a calibrated microphone (Microtech Gefell, WME 952), amplified (etec B1501) and stored on a laptop computer using a data acquisition card (National Instruments DAQ 6062 E) and a custom software package (SeaProDaq) (Pavan *et al.*, 2001) for further analysis.

RESULTS

POSITIONING OF ELECTRODES

The measurements to find the best placement for the electrodes revealed that the active electrode had to be placed at a position 2 cm forward of the ears on the dorsal midline (figure 4). The best position of the ground electrode was found to be 15 cm posterior to the ear line, while the best results were achieved with the reference electrode at a position 25 cm posterior to the ear line.

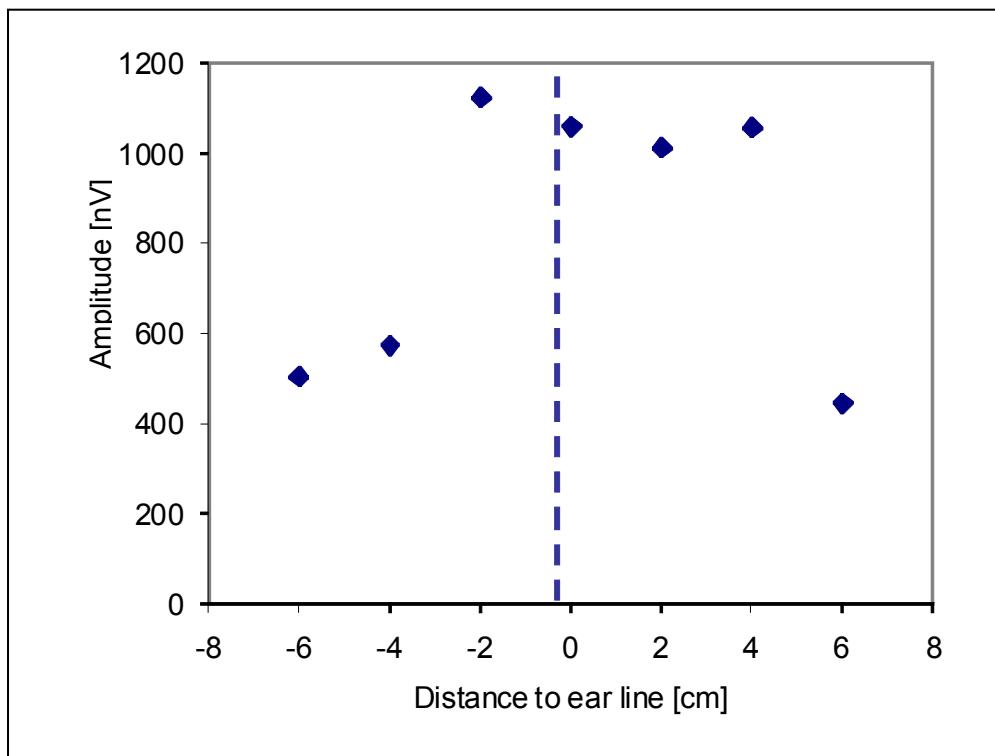


Figure 4. Peak-to-peak amplitude of AEP wave V (blue diamonds) measured in an adult harbour seal as function of the electrode position relative to the ear line (i.e. relative to ears on the dorsal midline). The ear line is indicated by the blue dashed line.

HEARING SENSITIVITY

Hearing thresholds were obtained from six harbor seal pups rehabilitated at the Seal Centre (figure 5, table 1). Thresholds were not measured at all pre-selected frequencies in all seal pups.

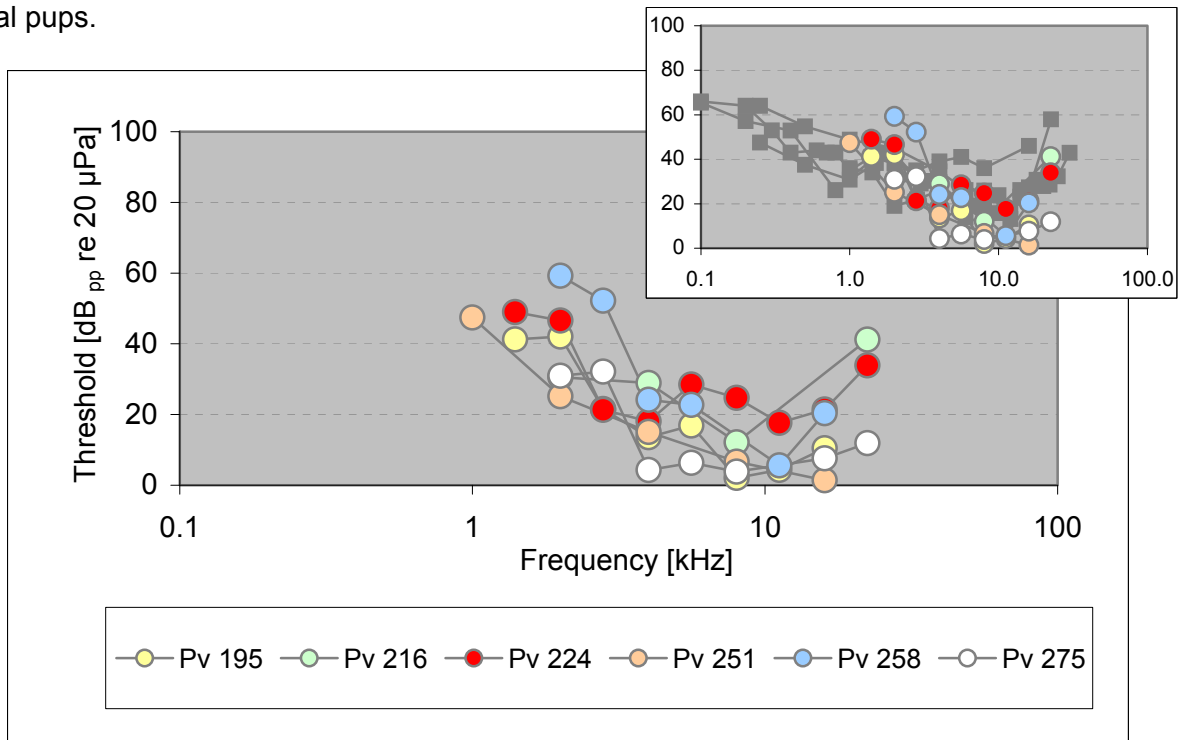


Figure 5. In-air hearing threshold data of six harbor seal pups (results from different individuals are color-coded) acquired by measuring neuronal responses (AEPs) to the repeated presentation of short cosine-gated sine waves. The measurements were conducted in quiet conditions and all animals were immobilized during the measurements. The small inserted figure shows the resulting thresholds for the harbor seal pups in comparison with hearing thresholds (gray symbols) acquired in behavioral auditory studies on adult harbor seals (see figure 1).

The six harbor seal pups showed a similar trend in their hearing curve, with comparable or even identical levels of hearing sensitivity at most frequencies. However, deviations of up to 35 dB were measured at frequencies below 2 kHz in some individuals. While the highest average levels were measured in the female seals Pv 224 and Pv 258, another female, Pv 275, had the lowest average hearing thresholds. Except for the lowest frequencies (0.7 kHz to 1.4 kHz), the audiograms of all six harbor seal pups showed a comparable trend and are generally in accordance with the published hearing threshold data from adult harbor seals (see inserted graph in figure 5).

Hearing threshold measurements were also conducted on four wild sub-adult harbor seals on sandbanks in the German and Danish Wadden Sea (figure 6 and table 1). As with the seal pups, hearing thresholds were not measured at all pre-selected frequencies.

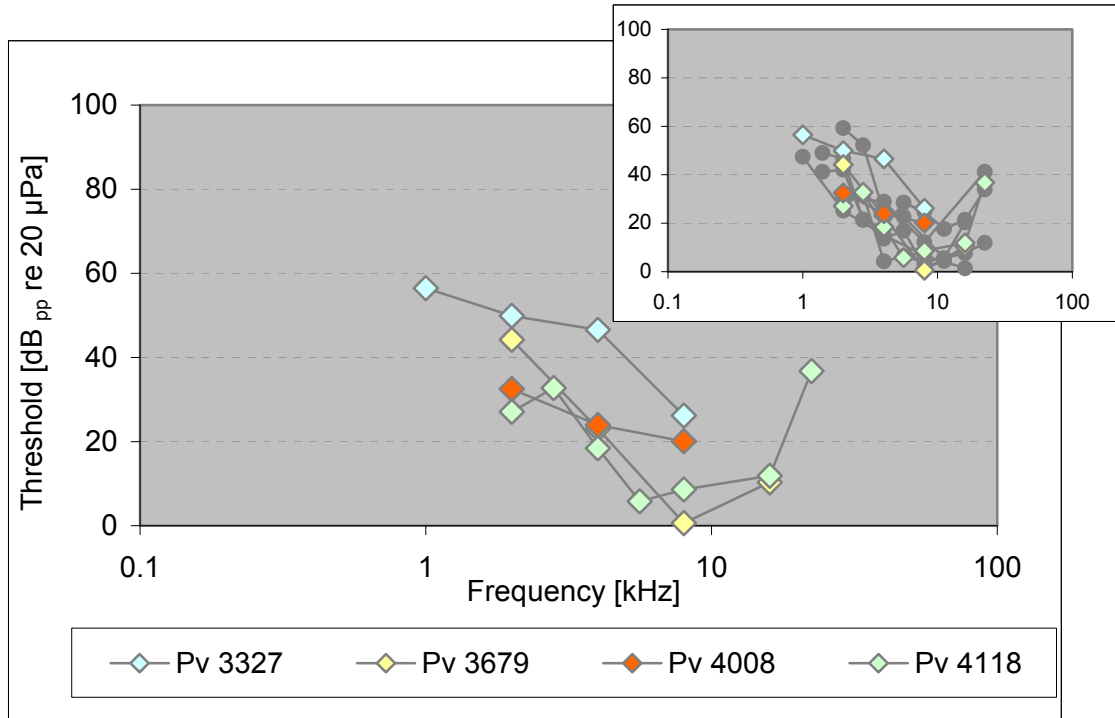


Figure 6. In-air hearing threshold data of four wild harbor seals (results from different individuals are color-coded) acquired by measuring neuronal responses (AEPs) to the repeated presentation of short cosine-gated sine waves. The measurements were conducted on sandbanks in the German and Danish Wadden Sea under quiet or moderate noise conditions, and all animals were immobilized during the measurements. The small inserted figure shows the resulting thresholds for the harbor seal pups in comparison with hearing thresholds (gray symbols) acquired in AEP studies on harbor seal pups at the Seal Centre in Friedrichskoog (figure 5).

Table 1. Hearing threshold data of six harbor seals tested at the Seal Centre Friedrichskoog, Germany, and of four wild harbor seals tested on sandbanks in the German and Danish Wadden Sea. Acoustic stimuli used to elicit AEPs at lower vs. mid- and higher frequencies differed with regard to their frequency bandwidth. The number of frequencies measured or yielding results differed between animals (missing data indicated by blank cells).

	Harbor seal pups						Wild harbor seals			
Animal (coded #)	Pv 195	Pv 216	Pv 224	Pv 251	Pv 258	Pv 275	Pv 3327	Pv 3679	Pv 4008	Pv 4118
Age [years] (*estimated)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1*	1*	1*	2*
Sex (m=male / f=female)	f	f	f	m	f	f	m	m	f	f
Frequency [kHz]	Hearing threshold [dB _{pk-pk} re 20 µPa]									
AEP-stimulus: Tone Pip / 8 cycles (Frequency bandwidth: 0.2 octaves)										
1				47.4			56.5			
1.4	41.2		49.0							
2	42.1	30.5	46.6	25.2	59.3	31.0	49.9	44.2	32.5	27.1
2.8	21.5		21.3		52.2	32.1				32.7
4	13.6	28.9	18.1	15.1	24.2	4.3	46.6	23.1	24.0	18.4
5.6	16.9		28.5		22.7	6.3				5.8
8	2.1	12.1	24.7	6.7		3.9	26.2	0.6	20.1	8.6
11.2	4.3		17.7		5.6					
16	10.5		21.4	1.4	20.4	7.6		10.3		11.9
22.4		41.2	33.9			11.9				36.8
AEP-stimulus: Tone Pip / 3 cycles (Frequency bandwidth: 0.8 octaves)										
0.7		22.8								
1.0	23.9		46.4		56.8	31.8				19.1
1.4					40.6	38.0				19.3

The data gathered from the wild animals cover the frequency range between 1 kHz and 22.4 kHz. However, only one animal yielded sufficient data to plot an almost complete audiogram of a free-ranging harbor seal. While the data from the four animals differ slightly, their variation is smaller than in the harbor seal pups tested at the Seal Centre. A visual comparison of the threshold levels from the wild harbor seals and the seal pups reveals no general difference.

In the harbor seal pups tested at the Seal Centre in Friedrichskoog, the best hearing sensitivity was measured at frequencies between 8 kHz and 16 kHz. The levels of highest sensitivity ranged between 1.4 dB_{pk-pk} re 20 µPa and 17.7 dB_{pk-pk} re 20 µPa in these animals. In the wild animals, best hearing sensitivity was documented at 5.6 kHz and 8 kHz with levels

between as low as 0.6 dB_{pk-pk} re 20 µPa and 26.2 dB_{pk-pk} re 20 µPa. While all harbor seals showed the same trend in their overall hearing curve, a strong variability was measured in different subjects. A maximum difference of 30.9 dB was found at 2.8 kHz in the seal pups. In the wild seals, the largest offset between individual thresholds of 25.6 dB was measured at 8 kHz.

These differences were even more pronounced with a maximum inter-subject difference of 32.9 dB measured at 1 kHz when tested at the low frequencies with stimuli of a wider acoustic bandwidth (3-cycles tone pips). The thresholds determined by using these wideband acoustic stimuli show an offset to those measured with the narrowband acoustic stimuli (8-cycles tone pips). The threshold variation between individuals is comparable to differences found in the threshold data from the published behavioral studies.

DISCUSSION

The results of this study show that the aerial hearing sensitivity of harbor seals from the North Sea is comparable to animals from other regions of the Northeast Atlantic and also the North Pacific. An auditory impairment is not detectable in the juvenile or sub-adult individuals tested within this study. Such an effect may accumulate over time due to long-term exposure to noise (e.g. the elevated level of anthropogenic noise in the North Sea) and become evident as a chronic state in adult harbor seals (see Kastak *et al.*, 2005a).

In this study, the AEP technique has proven to be suitable for testing both free-ranging and captive harbor seals and should be used in the future to increase the data basis on auditory sensitivity in this species. While a comparatively small number of animals was tested, the results indicate that the data from animals held temporarily in a controlled environment are comparable to those from wild animals.

The use of anesthetics to reduce myogenic artefacts and eliminate conscious muscular control over the outer ear canal (Kastak *et al.*, 2005b) was successfully applied in this study. Immobilization of the animals has proven to be a key element for successful in-air measurements of AEPs in untrained seals, but control of the acoustic environment is equally important in achieving reliable and reproducible data. Under immobilization, the outer ear canal remains open and headphones can be used to present the AEP-stimuli, thereby attenuating environmental noise by 12 dB. If conducted in a quiet environment (both indoors and on sandbanks), these measurement conditions therefore seem to be suitable for auditory testing in seals.

The intersubject variability in hearing thresholds may reflect existing individual differences in hearing sensitivity. However, an elevated background noise level (e.g. increased wind speeds) is likely to have caused masking of the acoustic stimuli at least during the measurements in one of the wild animals (Pv 3327). In both the wild seals and the seal pups at the Seal Centre, which were tested under a low and more uniform level of background noise, differences may be attributed to aspects of the immobilization:

- Ketamine, which was used as dissociative drug to immobilize the harbor seals, is known to cause a tremor in terrestrial animals (Erhardt *et al.*, 2004). The same effect was observed in some seals and may have thereby affected the AEP responses. A tremor elevates the neuronal background noise as any muscular activity, either controlled or unintentional, as in this case, is correlated with myogenic potentials. Even though the discrete myogenic potentials are strongly reduced relative to the auditory evoked potentials due to the averaging, they can nevertheless raise the neuronal noise floor, thus acting as an internal masker. The tremor can generally be reduced by carefully increasing the dosage of Diazepam or using narcotics to anesthetize the animals.
- Hearing thresholds were determined by extrapolating the dose-response function of received stimulus level vs. amplitude of neuronal wave V to the zero-level (Supin *et al.*, 2001; Popov *et al.*, 2007). As this function is not perfectly linear, any increase in neuronal noise could potentially reduce the accuracy of the resulting threshold value. Moreover, non-auditory potentials at higher voltage levels can directly interfere with the AEP response and change its shape, thus making it impossible to visually identify the individual waves of the AEP response. This effect can in principle be countered by increasing the number of averages which would prolong the procedure. As the number of averages is exponentially and inversely correlated with the absolute reduction in noise level, this measure is most efficient only at a low number of averages.
- The individual harbor seals also showed differences in their responsiveness to the dissociative. This resulted in a need for different intervals for administering the ketamine during the procedure. This may have caused an increased level of myogenic potentials interfering with the recorded neuronal responses, especially during the beginning of the procedure when a less sensitive animal was not fully immobilized.

Increasing the depth of anesthesia by using more potent narcotics would help resolve this problem by reducing neuronal noise. However, phocid seals appear very sensitive to narcotics and may show a dive response inherent with reduced life functions (heart rate, breathing) during deep anesthesia (Dierauf and Gulland, 2001). Therefore, all of the animals' life functions would have to be monitored during anesthesia, which would require several control devices. While this might be feasible at facilities like the Seal Centre, this approach is logistically challenging in the field. It has also to be taken into account that if the level of anesthesia is too deep, auditory processing is progressively inhibited from cortical levels down to the brainstem level where the AEPs measured in these animals originate (Hall, 2006). Nevertheless, further improvements of the auditory test procedure have to focus on the reduction of neuronal noise, and the use of alternative methods of anesthesia may be a sensible approach if conducted carefully.

An alternative approach is the use of a different acoustic stimulation. Instead of using discrete tone pips, the continuous presentation of an amplitude-modulated sine wave can elicits a continuous and frequency-specific brainstem response (Lins and Picton, 1995; John

et al., 1998). This method of measuring the so-called Auditory Steady State Response (ASSR) has been successfully applied in toothed whales (Dolphin, 1996; Finneran and Houser, 2007) and is currently being tested in some otariid (“eared”) seal species (Reichmuth, pers. comm.). ASSR allows for simultaneous testing of multiple frequencies. Even though it does not linearly reduce the total time needed, total testing time is likely to be shorter (John *et al.*, 1998). This would allow increasing the number of averages measured at each frequency and thereby reducing the neuronal background activity. Moreover, this method would potentially allow the measurement of threshold at frequencies at or below 1 kHz. It remains unclear whether acoustic stimuli used in this study at 1 kHz and below were effective in their entire length or only their beginning portion, as shown by Supin and Popov (1993). A wider effective bandwidth of the stimulus would result and hence an overestimation of the animals’ absolute hearing threshold at the frequency tested.

A comparison with data from behavioral studies (Møhl, 1968; Terhune, 1991; Kastak and Schusterman, 1998; Wolski *et al.*, 2003) and another AEP study (Wolski *et al.*, 2003) indicates that the thresholds determined in this study can be regarded as absolute thresholds – bearing in mind that the acoustic stimuli were different between these studies. However, Holt *et al.* (2001; see also Southall *et al.*, 2003) have shown that there can be a considerable influence of masking from background noise on the detection threshold in seals, resulting in an underestimation of their hearing sensitivity. As detailed information on the acoustic conditions during the previous hearing studies is not available, it is impossible to quantify whether or not the published data really represent the absolute hearing thresholds in harbor seals. Likewise, acoustic masking cannot be entirely ruled out in this study, even though the measurements were conducted in a quiet environment such as the Seal Centre’s medical room. However, the aim of the study was to assess the auditory health status of wild harbor seals and not to determine the ultimate hearing threshold levels for harbor seals. Therefore, it is more important that the acoustic conditions in both the captive and open environment were comparable. A comparison of the background noise level in both environments (with relative differences between the two being levelled out by the attenuation by the headphones) suggests that the results are indeed comparable.

Sandbanks represent the natural soundscape for harbor seals. This has to be taken into account for any assessment of the effects of anthropogenic sound on these animals. Consequently, the thresholds determined in this study under controlled conditions and in an open environment can be regarded as natural hearing threshold levels of harbor seals for in-air sounds.

Nevertheless, background noise and the animal's muscular activity remain limiting factors for such studies in general and should always be minimized as much as possible and carefully monitored and documented for comparison.

Adequate stimulus types and sound sources as well as electrode placement must also be carefully selected. The difference in duration of the presented acoustic stimuli is a factor requiring consideration in the comparison of threshold data from behavioral studies and AEP studies. The duration of stimuli used in AEP studies is usually in the range of less than ten milliseconds while stimuli in behavioral studies with seals are mostly several hundred

milliseconds or even seconds long. In normal mammalian hearing, acoustic perception improves (i.e. the threshold level decreases) if the duration of a signal increases up to the duration of the integration time of its hearing system (involving higher cortical levels which process the information). If stimulus durations fall below the integration time of the auditory system of seals, resulting thresholds would need to be corrected (i.e. reduced) by a factor of 3 dB per doubling of time. Holt *et al.* (2004) determined a temporal integration time of approximately 300 ms in a harbor seal for pure tones. If the integration for tone pips (used in this study) and pure tones would be comparable, this would result in a decrease of threshold levels by 15 dB to 27 dB. As this correlation has not been verified yet for the different types of acoustic stimuli and for a potential frequency dependence, threshold values should remain uncorrected.

The best positions for the cephalic electrode as established in this study are almost identical to those found by Reichmuth *et al.* (2007) in harbor seals.

An assessment of effects induced by anthropogenic noise would also require knowledge of the seals' hearing sensitivity to underwater sound, as most of the noise generated by anthropogenic activities is emitted underwater. So far the underwater hearing sensitivity of wild seals cannot be assessed with the AEP method.

However, the underwater frequency range of phocid seals (as the harbor seal) is extended to the higher frequencies compared to their in-air frequency range. Hemilä *et al.* (2006) concluded that the high-frequency hearing in these seal species may be constrained by ossicle inertia. This implies, that the sensitivity of the inner ear remains the same in air and underwater, only the transmitted sound energy and thus the resulting hearing thresholds and frequency range are different. Consequently, unless there are some anatomical pathologies which may interfere with the perception and transmission of underwater sound to the inner ear, an assessment of the health status of harbor seal low- and mid-frequency hearing can be made based on in-air hearing capabilities. Any morphological or pathological anomalies should occur on an individual level rather than a population level. Monitoring the in-air sensitivity of a wild population should therefore provide sufficient information to assess potential long-term changes in the auditory health status of harbor seals. Due to the individual variability in hearing sensitivity as determined in this study, it is evident that initially only trends in hearing sensitivity can be identified.

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PERCEPTION OF LOW-FREQUENCY ACOUSTIC SIGNALS BY A HARBOR PORPOISE (*PHOCOENA PHOCOENA*) IN THE PRESENCE OF SIMULATED OFFSHORE WIND TURBINE NOISE

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ABSTRACT

Using auditory evoked potential (AEP) methods, a study was conducted on a harbor porpoise (*Phocoena phocoena*) at the Dolfinarium Harderwijk in The Netherlands. The study measured the audible range of wind turbine sounds and their potential masking effects on the acoustic perception of the animal. AEPs were evoked with two types of acoustic stimuli: (1) click-type signals and (2) amplitude-modulated signals. The masking noise resembling the underwater sound emissions of an operational wind turbine was simulated. At first, the animal's hearing threshold was measured at frequencies between 0.7 and 16 kHz. Subsequently, these measurements were repeated at frequencies between 0.7 and 2.8 kHz in the presence of two different levels of masking noise. The resulting data show a masking effect of the simulated wind turbine sound at 128 dB re 1 μ Pa at 0.7, 1.0 and 2.0 kHz. This masking effect varied between 4.8 and 7.3 dB at those frequencies. No significant masking was measured at a masking level of 115 dB re 1 μ Pa. The available data indicate that the potential masking effect would be limited to short ranges in the open sea, but limitations exist to this conclusion and all estimates are based on existing turbine types, not taking into account future developments of larger and potentially noisier turbine types.

Key Words: harbor porpoise, *Phocoena phocoena*, acoustic perception, masking, auditory evoked potentials, North Sea, offshore wind turbines

INTRODUCTION

The harbor porpoise (*Phocoena phocoena*) has become a subject for intense studies over the last decade because of numerous threats for this species and the lack of knowledge in many aspects of its natural history. Many of these threats are of an acoustic nature. Since harbor porpoises were shown to be very sensitive to different types of acoustic signals (Thompson, 2000; Cox *et al.*, 2001; Bain & Williams, 2006), there is growing concern about the behavioral and physical effects as well as their implications for the individuals, respectively, and the porpoise populations in ensonified areas. Recently, an additional source for acoustic emissions is being introduced into their environment by the planned installation of wind turbines in offshore areas. Many projects are currently under consideration in several European countries to build units in the offshore areas of the North and Baltic Seas, hence spatially overlapping with the distribution of harbor porpoises. To date, these plans comprise the installation of several thousand wind turbines. During the operational phase, the wind turbines emit low-frequency, continuous noise via vibrations of the pilings.

A principal key for assessing the impact of these noise emissions on the harbor porpoises is the understanding of the auditory sensitivity and perception capabilities of this species. In harbor porpoises, like in several other cetacean species, the auditory sense evolved to be the primary sensory modality. This is not only represented by the sophisticated sound production mechanism, as seen especially in odontocete cetaceans (Amundin, 1991), but also by the auditory capabilities of these animals. Harbor porpoises are proven to actively use underwater sound by means of echolocation (Busnel *et al.*, 1965; Møhl and Andersen, 1973; Akamatsu *et al.*, 1994) to obtain considerable information about their environment for a number of different purposes (e.g. detection of food, obstacles, predator avoidance and navigation). Also, the auditory sensitivity of the harbor porpoises has been studied by Andersen (1970) and Kastelein *et al.* (2002), each obtaining behavioral audiograms of a single captive harbor porpoise. Bibikov (1992) obtained electrophysiological audiograms from three harbor porpoises by measuring the auditory evoked potentials (AEPs). Harbor porpoises hear at least between 250 Hz to 160 kHz. Their most sensitive hearing range (32 dB re 1 μ Pa is at 100 to 140 kHz) (Kastelein *et al.*, 2002) overlaps the frequency content of their echolocation clicks (i.e. between 125 kHz and 148 kHz) (Møhl & Andersen, 1973; Hatakeyama & Soeda, 1990; Goodson *et al.*, 1995). Towards the lower frequencies, their acoustic sensitivity drops consistently, reaching 92 dB re 1 μ Pa at 0.5 kHz.

The AEP method is one of the methods commonly used to collect audiometric data from humans, as well as animals, and is increasingly used also for studying the auditory system of marine mammals. This electrophysiological method allows measuring hearing in a comparatively short period of time. The presentation of acoustic stimuli generates neuronal potentials in the auditory system (i.e., AEPs) upon perception of these stimuli. These potentials are generated within neuronal nuclei at different positions in the auditory system thereby forming an electrical field, which can be detected and recorded even on the skin surface – the electrical far-field. This so-called electrical far-field of the potentials is not evenly distributed on the body surface. The strongest potentials, and thereby the ideal

position for the active electrode, is species-specific and has to be established prior to the auditory measurements.

The typical AEP of a harbor porpoise, similar to that of a bottlenose dolphin (*Tursiops truncatus*), has three positive and two negative peaks with increasing amplitudes, but the harbor porpoise waves have longer latencies than those in bottlenose dolphins (Bullock *et al.*, 1968; Ridgway *et al.*, 1981; Bibikov, 1992; Supin *et al.*, 2001).

A refined methodological approach is based on the use of rhythmic sound modulations. By sinusoidally modulating the amplitude of a carrier tone or sound pulse sequence, it is possible to elicit a neuronal response, which includes a specific frequency component that is equivalent to the modulation frequency of the stimulus, an effect called envelope-following response (EFR). By applying a fast Fourier transformation (FFT) analysis, the modulation frequency component can be identified and quantified. The resulting amplitude of the EFR represents the energy content of the neuronal response at the given modulation frequency. Nevertheless, the strength of this EFR response can simultaneously be taken as a relative measure for the perception of the carrier frequency of the amplitude-modulated (AM) signal because no EFR response could be elicited if the signal was not within the functional and dynamic range of the auditory system. Usually, the EFR begins with a delay of a few milliseconds after the stimulus onset and lasts for at least 10 ms. In general, a period of 16 ms (e.g., between 7 and 23 ms) was analyzed (Supin *et al.*, 2001).

The advantage of this AM approach is its higher-frequency specificity. While EFR can be attributed to a specific frequency, short-pulsed signals have a broader frequency spectrum in general and thus represent a measurement of the auditory sensitivity over a wider frequency range. On the other hand, a lower frequency limit exists for the use of AM signals. Auditory measurements at low frequencies therefore are preferably conducted using short-pulsed signals.

The perception of a noisy sound by a harbor porpoise might simply be ignored by the animal. With an increase in the received sound pressure levels (SPLs) of noise, the perception might increase stress for the animal and result in a behavioral reaction or interfere with the perception of biologically important sounds. The detection of a signal by a marine mammal's ear, in general, can be affected by interference from noise in frequency bands near that signal (e.g., Erbe and Farmer, 1998; Finneran *et al.*, 2002). This effect is called masking, and whether or not such masking occurs depends on the acoustic characteristics of a sound, its received level and the acoustic sensitivity and characteristics of the receiver's hearing system.

Sounds associated with wind turbine operation have their main acoustic energy in a low-frequency band and show strong tonal components at type-specific frequencies below 1 kHz, thereby overlapping with the hearing range of the harbor porpoise. The aim of the present study was to assess the range of audibility of wind turbine signals for the harbor porpoise, as well as the amount of masking effect for these animals – that is, the impaired or impeded perception of signals in the presence of wind turbine acoustic emissions.

MATERIALS AND METHODS

SUBJECT AND FACILITY

The auditory measurements were conducted on a stranded male harbor porpoise (code PpSH047) kept at the Netherlands Cetacean Research and Rehabilitation Centre (SOS Dolfijn) at the Dolphinarium Harderwijk, Harderwijk, The Netherlands. This animal (“*Daan*”) stranded in 1997 and was approximately 7 to 8 years old at the time of the research. His body length was 136 cm and his weight was 38 kg. *Daan* was previously trained to participate in psychophysical studies and was also the study animal for the measurement of the behavioral audiogram by Kastelein *et al.* (2002).

Daan was kept in an oval-shaped concrete indoor pool at the Dolphinarium Harderwijk. The dimensions of the research pool were 8.6 m by 6.3 m, with an average water depth of 1.5 m. During the research, the water level was kept constant (± 5 cm) and there was no strong current in the water during the measurements because all pumps were switched off before the start of any session.

SET-UP AND BEHAVIORAL PROCEDURES

The underwater station at which the animal was trained to station for the audiometric measurements was placed at a depth of 0.75 m (mid-water) in the research pool. This station was made of a plastic ball-shaped mesh (3 cm diameter) attached to a PVC pipe hanging vertically from a pole lying across the pool. The three transducers used for transmitting the AEP stimuli were positioned at a fixed distance of 1 m to the animal’s rostrum of 1 m at a depth of 0.75 m.

Due to the small size of the research pool and its shape, every acoustic stimulus emitted into the research pool was subject to constructive and destructive interferences due to multi-path reflections from any boundary within less than a millisecond. Two acoustic reflecting baffle-boards were installed 0.4 m from the hydrophones (figure 1) to prevent the animal from receiving the direct sound reflections off the water surface and pool bottom. Both baffle-boards (width: 1.2 m; effective height in the water: 0.3 m each) consisted of cork-loaded neoprene tiles attached to a wooden plate. One baffle-board was positioned at the bottom of the pool, the other one was positioned at the water surface, and both were perpendicular to the direct sound path. A monitoring hydrophone was placed in front of the underwater station of the animal. Additionally, all sessions were visually monitored via a digital video camera installed overhead, and the video signals were monitored on a video screen near the primary researcher.

Any external noise which could potentially mask the stimulus was reduced as far as possible by interrupting all noise-related activities and switching off all machines in the rehabilitation center during the conduct of the measurements.

Daan was trained to voluntarily participate in the measurements (i.e., to accept being equipped with the sensors and to subsequently swim to an underwater station where he positioned himself and was exposed to the acoustic stimuli). The animal had to continuously station there for at least 25 s as motionless as possible. His body axis was positioned in a direct line with the transmitting source. Upon the presentation of a bridging stimulus, *Daan* returned to the poolside start position to receive reinforcement (fish) and paused before repeating the procedure. Each of these behavioral sequences was called a trial, and a full research session consisted of one up to five trials.

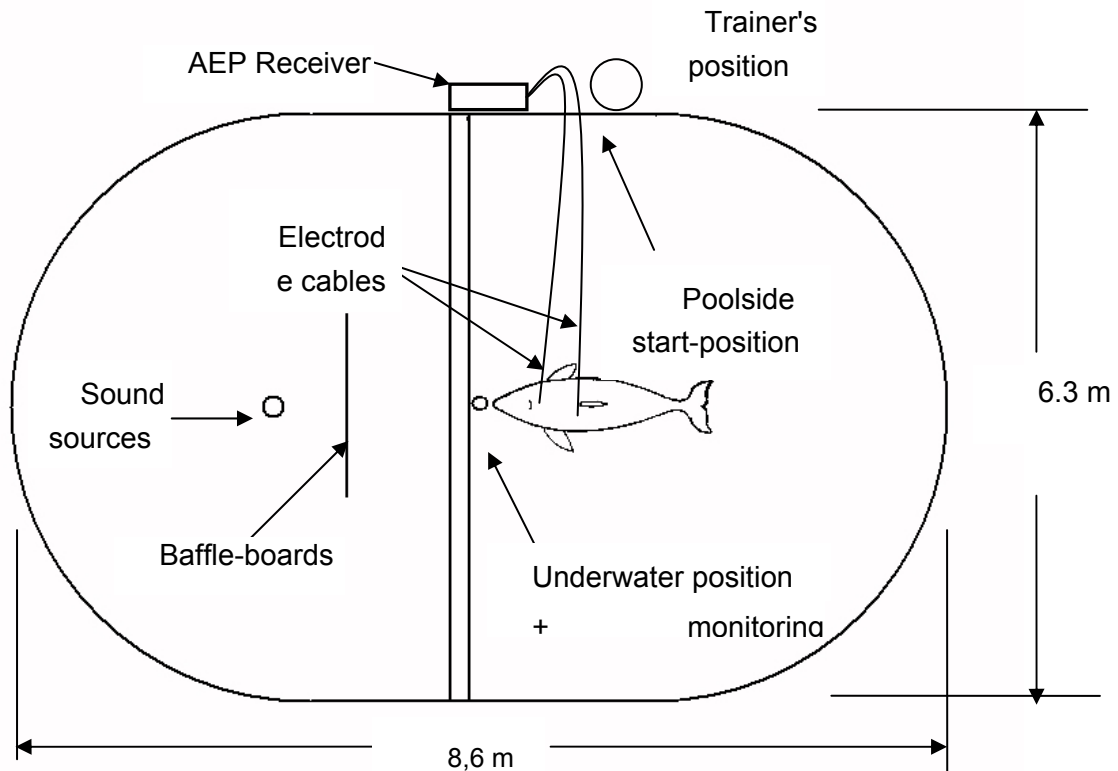


Figure 1. Schematic overhead view onto research pool and set-up with the animal's position at its underwater station indicated

EXPERIMENTAL PROCEDURES

Methods – Measurements were conducted using the AEP method. Two surface electrodes (active and reference electrode) were placed on the animal's skin using suction cups – one near the blowhole and the other near the dorsal fin – to record the neural responses evoked by the auditory system.

Stimuli and Frequencies – The hearing threshold measurements were conducted at frequencies ranging from 0.7 kHz to 16.0 kHz, thus covering a range in which masking from the operational noise of the wind turbines could theoretically occur. Subsequently, measurements were repeated in the presence of the simulated wind turbine sounds emitted at two intensities. Starting from 0.7 kHz, this procedure was repeated at all higher

frequencies in an ascending order until no masking was detected. Based on statistical considerations, these measurements had to be repeated several times at each of these five frequencies and at two masking sound intensities.

At frequencies between 2.0 kHz and 16.0 kHz, AM signals were used; while at < 2.0 kHz, a short pulsed signal (tone pip) was taken as the stimulus (thus providing a frequency overlap at 2.0 kHz for comparison). This stimulus (figure 2) lasted for 1.5 cycles of the test frequency and was cosine-gated over 0.1 ms. All AM signals were fully modulated (modulation depth = 100%) and had a duration of 25 ms with a rise/fall time of 0.5 ms. All signals were transmitted at varying levels, starting at clearly audible levels and subsequently decreasing SPLs at 5-dB intervals in a series of descending runs. All signals were alternated in phase by 180° between successive stimuli.

Masking Sound – Meanwhile, several underwater acoustic measurements of sounds emitted from a wind turbine were sampled in Sweden, Denmark and Germany (Westerberg, 1994; Degn, 2000; Fristedt *et al.*, 2001; Ingemansson Technology AB, 2003; Betke *et al.*, 2004; see also Wahlberg & Westerberg, 2005, and Madsen *et al.*, 2006, for reviews). They all differed with regard to the type and size of wind turbine, its foundation, the bottom sediment and water depth, as well as the weather and wind conditions. Nevertheless, in general, sounds associated with wind turbine operation have their main acoustic energy in a low-frequency band and show strong tonal components at type-specific frequencies below 1 kHz. Therefore, a masking sound was simulated resembling the noise of an offshore wind turbine over a frequency spectrum from 16 Hz up to >1 kHz, with strong tonal components at 200 Hz and 500 Hz.

The simulated operational noise was continuously transmitted during the masking experiment at two varying levels: moderate (m_1) and high (m_2), with the high level reaching a maximum received level at 200 Hz of 128 dB re 1 μ Pa at 1 m (see figure 3) and the moderate masking level at a maximum level of ~115 dB re 1 μ Pa at 1 m.

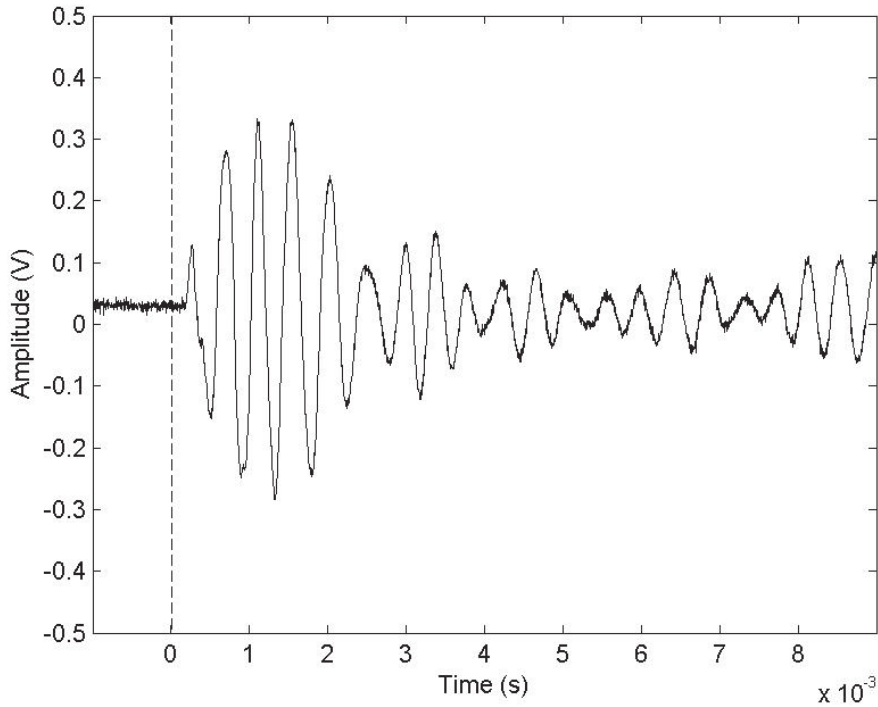


Figure 2. Waveform of the received signal of a 2-kHz Ricker type pulse at 1-m range; the small inserted figure shows the 2-kHz tone pip as an outgoing drive signal in comparison.

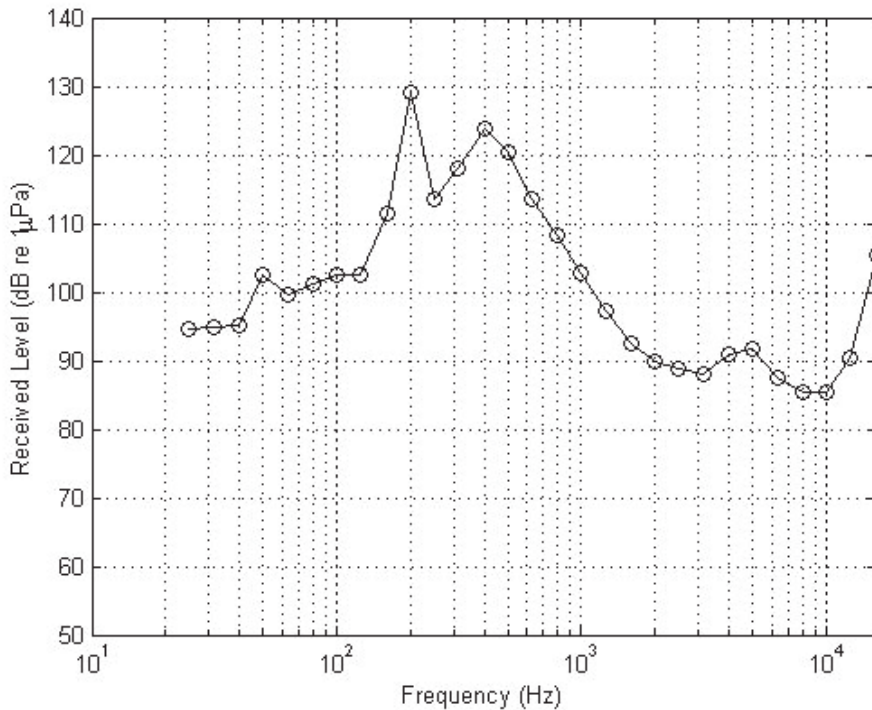


Figure 3. Frequency spectrum of simulated operational sound of an offshore wind turbine; the received levels (third-octave analysis) represent the high masking noise level used during the masking experiment.

STIMULUS GENERATION AND PRESENTATION

All AEP stimuli up to 16 kHz were generated by using the software SigGen (Tucker-Davis Technologies [TDT], Alachua, FL, USA). The stimuli were then fed into a TDT Workstation, System 3, at a digitization rate of 50 kHz, subsequently amplified by a Brüel & Kjær amplifier 2713 and transmitted via a hydrophone into the water (0.7 kHz to 2.0 kHz: SRD ball hydrophone [10.16-cm diameter], 2.8 kHz to 16.0 kHz: Reson TC 4033). The emitted signals were constantly monitored via a hydrophone (Reson TC 4014) installed in front of the animal, amplified by a custom-made pre-amplifier (etec B1501). All signals were recorded onto a hard disk drive.

During the experiment, the masking sound was constantly played from a laptop computer, with a power amplifier (PA 100E, Ling Dynamic Systems Ltd., Royston, UK) and transmitted via an underwater transducer (USRD J-9). All AEP stimuli were presented to the animal at a rate of 20 signals/s and were monitored on an oscilloscope simultaneously. The hydrophones were calibrated before, during and after the study. Furthermore, the calibration factor of all other devices used in the experiment was checked thoroughly and taken into account for the analysis in the frequency range from 0.7 kHz to 160.0 kHz.

Impulsive Sounds (0.7 kHz to 2.0 kHz) – The analysis of the recorded low-frequency sound stimuli showed a strong tonal component close to the natural resonance of the transducer (SRD ball hydrophone) at 2.0 kHz. The extension of the signal shown in figure 4 is due to the transducer being driven at a frequency close to its natural resonance causing the transducer to ring. This stretching of the pulse has resulted in the generation of a strong relatively narrowband tonal around 2.0 kHz shown in figures 5 and 6. Analysis of similar signals of 1.5 cycles at 1.4 kHz, 1.0 kHz and 0.7 kHz generated strong resonance effects at the transducer natural frequency. These effects were seen as impulsive transients with an energy of 5 dB below the maximum value. Such impulsive signals at the beginning and end of the pulse form a relatively broadband spectral response. The impulses are then joined with multi-path arrivals at the receiver, resulting in an extended relatively broadband (1.0 to 8.5 kHz) signal generation at lower-received levels than center frequency.

Amplitude-Modulated Stimuli (2.0 kHz to 16.0 kHz) – The transmitted AM signals (e.g., 8.0 kHz, see figures 7 and 8) showed no on- or off-set response of the hydrophone. Due to their duration of 25 ms, they were subject to constructive and destructive interference from multi-path signals after < 1 ms. As the degree of distortion varied over the period of each trial between successive signals, the averaged received stimuli were less affected and retained their acoustic characteristics. Sidebands were observed as tonal components at frequencies 1.2 kHz above and below the carrier frequency, thus representing the frequency difference between carrier and amplitude-modulation frequency (correspondingly, the frequency difference was 0.7 kHz at a carrier frequency of 2.0 kHz). These artefacts occurred at levels 10 dB below the carrier frequency signal on average, however.

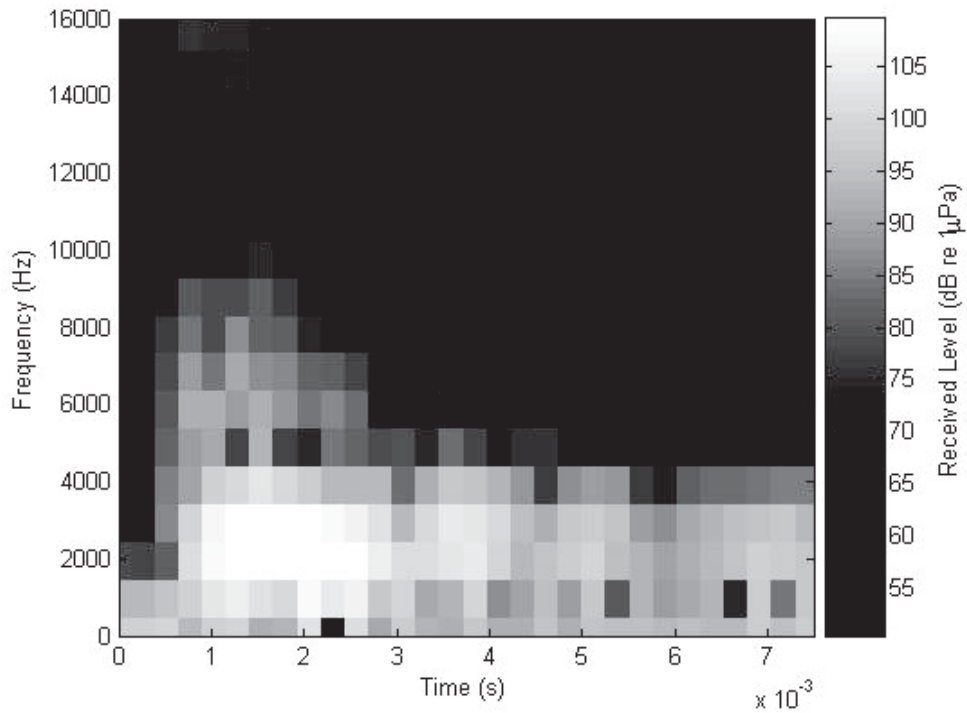


Figure 4. Spectrogram of the 2-kHz Ricker type pulse; strong tonal component due to transducer natural resonance effects.

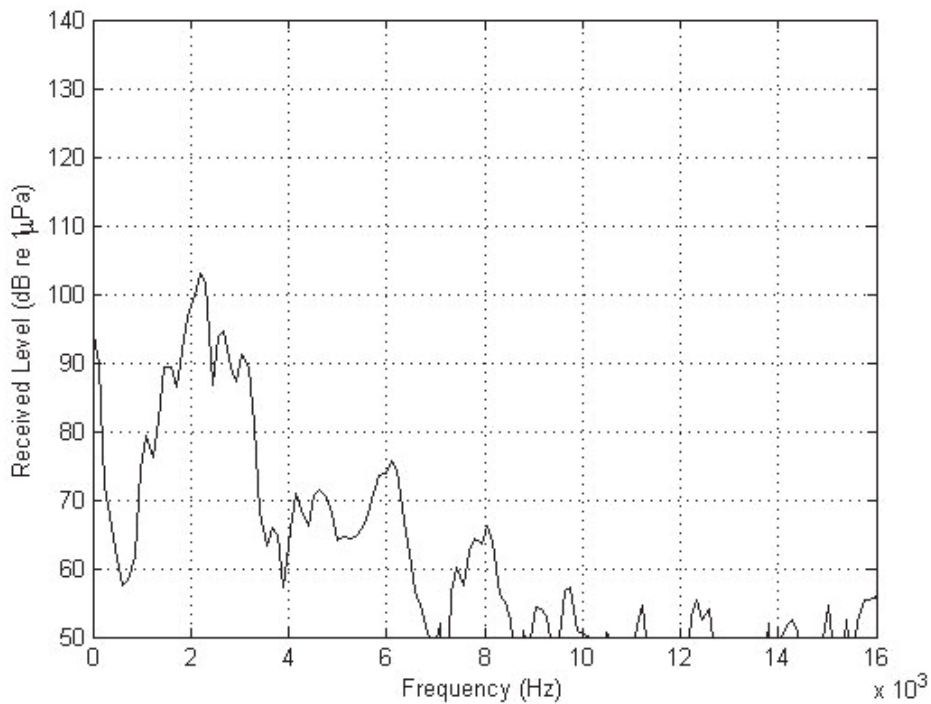


Figure 5. Spectral response for 8.2-ms analysis window, including multi-path signals; spectrum levels based on a 125-Hz analysis band.

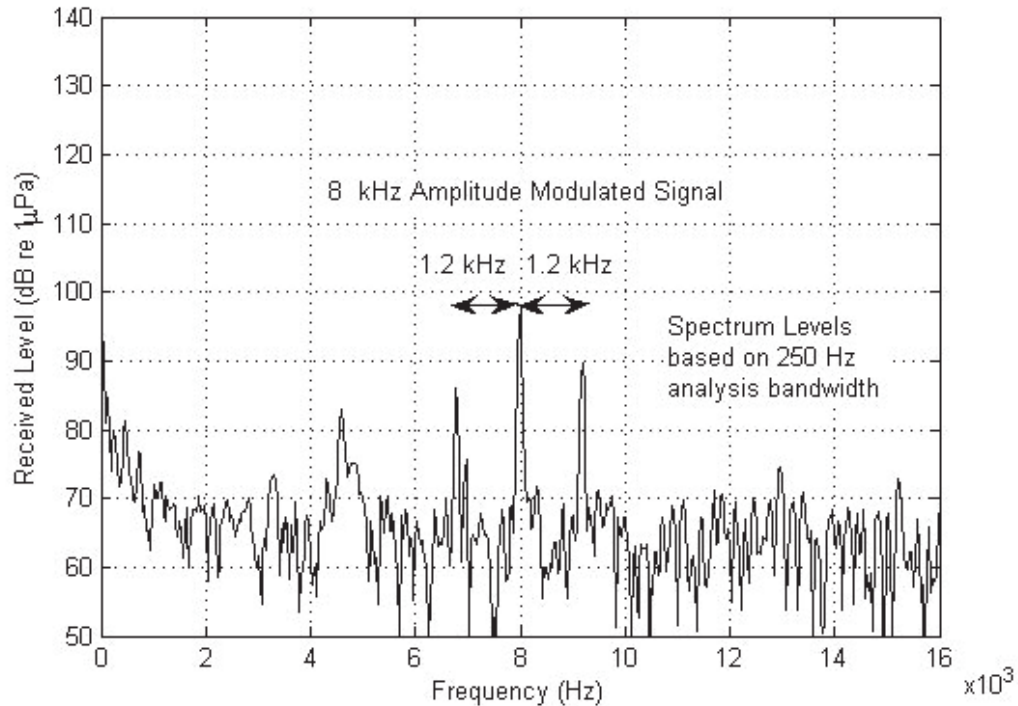


Figure 6. Frequency spectrum of the 8-kHz amplitude-modulated stimulus; two distinct signal components exist at 1.2 kHz below and above the stimulus, representing the hydrophone response to the stimulus modulation at a frequency of 1.2 kHz. The additional dominant component at 4.5 kHz is a result of internal noise and was not transmitted into the water.

BACKGROUND NOISE

The background noise in the research pool was recorded while no animal was in the water. The average background noise level in the research pool was about 55 dB re 1 $\mu\text{Pa}^2/\text{Hz}$. Only at 50 Hz is a high-energy amplitude present, which can be attributed to either internal electric noise in the recording system or transmission of this signal into the water.

RESPONSE ACQUISITION

The electrode responses (active and reference) served as input to a low noise differential amplifier (TDT RA4L; 20 dB gain). The amplified analogue signal was then passed through an anti-aliasing filter and led to an A/D converter (TDT RA16). The response (digitisation rate: 25 kHz) was digitally filtered, written to a memory buffer, tested for the presence of unwanted signal artifacts (artifact reject level: 80%) and added to the buffer containing the responses to the previous n stimulus presentations. The evoked responses were recorded and averaged over 500 recordings to acquire one data set. On average, five of these data sets were acquired at each intensity level for each frequency. All responses were bandpass-

filtered between 500.0 Hz and 2.0 kHz. Examples of the acquired AEP evoked by using tone pips as well as the AM stimuli are presented in figures 9 and 10.

Mapping the Porpoise Head – Prior to the audiometric measurements, the optimum position of the active electrode was established by changing its position along the dorsal bodyline of the porpoise. A clearly audible AM stimulus was used at a constant, supra-threshold SPL to elicit neuronal responses. These tests were done at a distance of 3.0, 6.0, 7.5, 9.0 and 12.0 cm behind the blowhole. The strongest EFRs resulted from measurements with the active electrode placed at a distance of 7.5 cm behind the animal's blowhole (figure 11). Consequently, this position was chosen as the ideal position for the suction cup. The reference electrode was placed near the dorsal fin, approximately 40 cm from the blowhole.

Modulation Rate Transfer Function (MRTF) – The auditory system showed qualitative differences in its ability to follow the envelope of a sinusoid. To identify the best frequency to modulate, the amplitude of the carrier signal, the so-called modulation rate transfer function (MRTF) was established prior to the auditory measurements. An MRTF was acquired using different frequencies to modify a carrier signal of 20 kHz. The resulting MRTF revealed three modulation frequencies with almost identical response amplitudes for the EFR: at 0.7, 0.9 and 1.2 kHz (figure 12). All three frequencies could be used to modulate the amplitude of the carrier signal and would result in the highest achievable EFR amplitudes. Thereby, the lower (0.7 kHz) allowed the lowest possible carrier frequency to be tested and the highest (1.2 kHz) provided the best signal-to-noise ratio of the EFR responses. For the latter reason, 1.2 kHz was chosen as the best modulation frequency for the AM stimuli in this study, except for 2.0 kHz where 0.7 kHz was used as the modulation frequency instead.

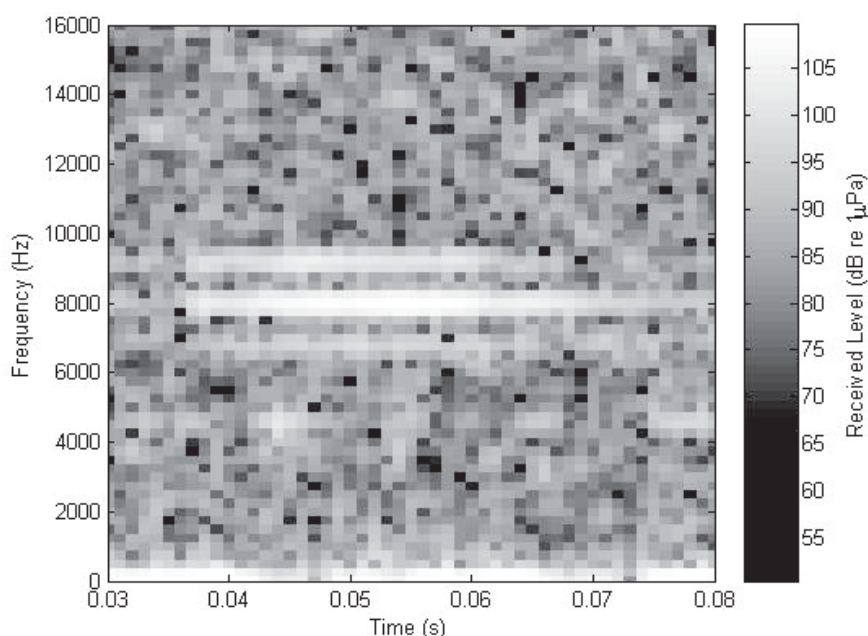


Figure 7. Spectrogram of the 8 kHz amplitude-modulated stimulus over a period of 50 ms; pulse duration is 25 ms. The received levels are color-coded on a gray scale, with white being the most intense and black the least intense. Two signal components can be identified at 6.8 kHz and 9.2 kHz, representing the hydrophone response to the stimulus modulation at

1.2 kHz. Further dominant signal components are related to the receiving system and have not been transmitted into the water.

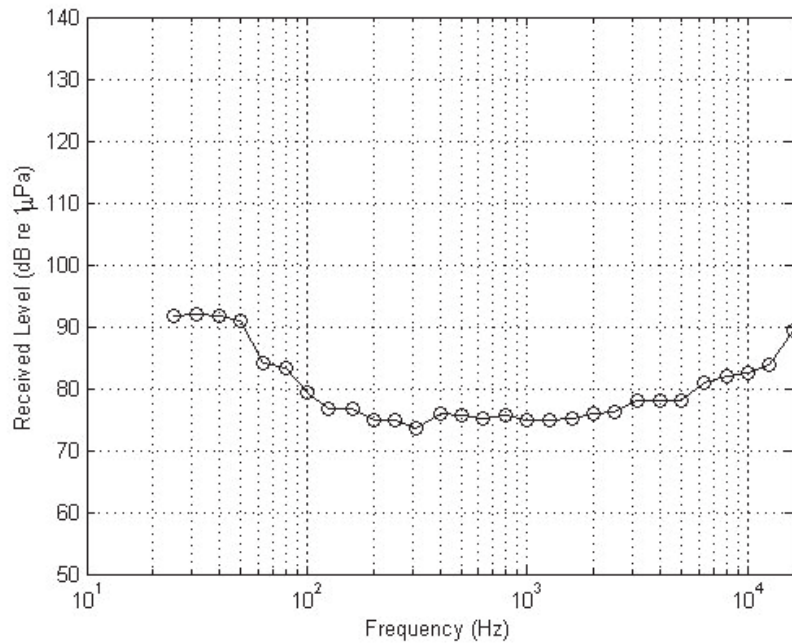


Figure 8. Background noise spectrum analyzed in third-octave steps

DATA ANALYSES

Pulsed Stimuli – Useful data sets (i.e., curves of 500 AEP responses) were identified by first obtaining an idealized response curve for every frequency at every masking level. Those were achieved by cross-correlating the data sets with a best-fit curve (visually selected from data sets measured under optimum conditions, e.g., with regard to the animal’s behavior and lowest level of disturbing external factors), thereby determining their cross-correlation coefficient and the lag at which it occurred. Subsequently, the measured curve was considered as a response if its cross-correlation coefficient was in excess of a certain threshold and its lag was smaller than a given threshold. Corresponding thresholds were determined by cross-correlating the idealized response curves with controls (i.e., “response” curves measured without prior stimulation) and then cutting off the smallest 60% (corresponding to the lowest correlation coefficient of the best-fit curves) of the obtained cross-correlation coefficients and the largest 95% of the absolute lags. The remaining curves were further analyzed by measuring the peak-to-peak amplitude of the most prominent and most consistently detectable potential and applying a linear regression to the resulting data of amplitude response as a function of stimulus level. The zero-crossing of the regression function represented the hearing threshold for the given frequency. To associate 95% confidence limits with the determined hearing thresholds, these data were subsequently bootstrapped with 1,000 samples. The data were tested for significance to verify the relationship between the received level of the acoustic stimuli and the evoked neuronal response. This analysis was applied to the data sets achieved by using tone pips between

0.7 kHz and 2.0 kHz as acoustic stimuli and under the different conditions (no masking, moderate, and full masking). This complex statistical analysis allows the declarative strength of the calculated threshold values to be assessed and was done for all tested frequencies irrespective of the masking condition.

Amplitude Modulated Stimuli – An FFT was applied to all data sets achieved by using AM signals as acoustic stimuli (i.e., between 2.0 kHz and 16.0 kHz). The magnitude of the EFR was assessed in the frequency domain by observing the amplitude of the spectra at the frequency of the amplitude modulation. By applying an F-Test to the resulting FFT data, those EFR responses were identified, which were significantly different from any electrophysiological background noise being present in the AEP recordings. Two methods exist for determining the hearing threshold value from the remaining EFR data. One is a regression analysis on all resulting EFR amplitudes to identify the zero-crossing and hence the threshold value. A second method (Supin *et al.*, 2001) includes a visual analysis of data prior to a regression analysis. This method is based on the fact that the EFR values at the frequency of amplitude modulation ideally decrease clearly from high values at supra-threshold stimuli levels to medium values at lower intensities. The values would remain constant over a certain range of stimulus intensities, thus forming a plateau, if plotted graphically, before they finally decreased at stimulus intensities near the threshold. Only the EFR values of the latter intensity range were analyzed in this study by using a regression analysis and thus provided a very precise estimate of the hearing threshold. This approach required EFR data of high signal-to-noise ratio, however. As soon as the recorded AEP responses are disturbed by other neuronal activity, a clear distinction of the final phase becomes increasingly difficult. Both types of analysis were applied to the available data in this study, where applicable. For both methods, the 95% confidence limits of the resulting threshold values were calculated by bootstrapping over 1,000 samples.

RESULTS

IMPULSIVE SOUND STIMULI

At 2.0 kHz and below, tone pips were used as acoustic stimuli for measuring *Daan's* auditory sensitivity and the potential masking effect of wind turbine-related operational noise. The threshold values and 95% confidence limits as listed in table 1 were determined after a statistical analysis and filtering of these data.

Table 1. Hearing threshold values of a harbor porpoise and statistical results for three frequencies tested with tone pips as acoustic stimuli at three different levels of masking noise (m_0 = no masking, m_1 = moderate masking and m_2 = high masking level); the threshold values are given along with 95% confidence limits, information on significance (** = highly significant) and further statistical information. Significance reflects the relationship between the received level of the acoustic stimuli and the evoked neuronal responses. The number of data points included in the analysis is given for every frequency. Numerator DF are always 1.

Frequency	Masking level	Lower confidence limit	Threshold	Upper confidence limit	F	Error DF	Significance	Number of values	
[kHz]	m_{0-2}	[dB re 1 μ Pa]							
0.7	0	50.8	60.8	65.3	52.81	46	0	**	48
0.7	1	60.5	64.8	68.1	82.29	24	0	**	26
0.7	2	69.1	72.1	74.8	82.2	25	0	**	27
1	0	-9.4	25.2	43.0	9.76	46	0.003	**	48
1	1	10.0	39.7	54.0	12.58	48	0.001	**	50
1	2	33.8	47.8	56.6	21.05	36	0	**	38
2	0	62.1	69.9	71.3	49.62	34	0	**	36
2	1	46.8	64.8	74.7	15.24	17	0.001	**	19
2	2	74.5	78.6	81.1	55.85	19	0	**	21

Data for the measurements at 1.4 kHz in the presence of high masking levels revealed an insignificant and nonrealistic threshold value, as well as confidence limits. All other threshold values were significant ($p < 0.01$). Nevertheless, the resulting threshold values varied considerably between 25.2 dB at 1.0 kHz and 78.7 dB at 2.0 kHz. At 0.7 and 1.0 kHz, the threshold values increased with increasing masking level, while at 1.4 and 2.0 kHz, the threshold values at moderate masking intensity were below the unmasked threshold. At 2.0 kHz, however, the high masking level led to a substantial increase of the threshold as compared to the unmasked situation. Most importantly, the threshold value at moderate masking conditions was within the confidence limits for all four frequencies. In contrast, the threshold values measured in the presence of high levels of masking sound were outside the confidence limits for 0.7, 1.0 and 2.0 kHz. The threshold value achieved at a high level of masking noise was 6.8 dB above the upper confidence limit of the unmasked threshold values at 0.7 kHz, 4.8 dB above at 1.0 kHz and 7.3 dB above at 2.0 kHz.

AMPLITUDE-MODULATED STIMULI

Threshold and Masking Analysis – The analysis, as used for example, by Supin *et al.* (2001), included only those data which were visually identified as being close to the threshold. The results for the masking experiment at 2.0 and 2.8 kHz are listed in table 2(a) for all three masking conditions. Threshold values were also calculated for the remaining frequencies for an unmasked condition; the results for all unmasked results are listed in table 2(b).

All data sets included in the regression analysis revealed significant ($p < 0.05$) results. At 2.0 kHz, the threshold differed only slightly between the three masking levels, with the lowest level analyzed for moderate masking. The thresholds achieved in the unmasked and masked conditions were almost identical. At 2.8 kHz, the threshold slightly increased from the unmasked to the moderate condition, while the threshold established for the high masking level was almost 10 dB below the unmasked threshold value.

The threshold values achieved by analyzing only the visually selected data sets in an unmasked condition between 2.0 and 16.0 kHz showed only a moderate variation, ranging from 59.4 dB_{rms} re 1 μ Pa at 5.6 kHz up to 85.0 dB_{rms} re 1 μ Pa at 16.0 kHz – a range of 25.6 dB. With an exception at 2.0 kHz, the confidence intervals are comparatively narrow.

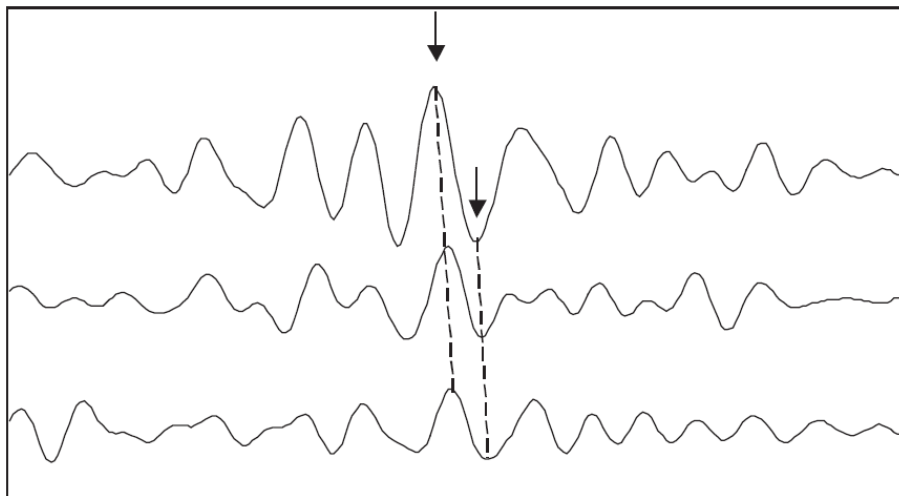


Figure 9. Examples of potentials evoked with tone pip in the harbor porpoise; sampling duration was 10 ms and center frequency of the stimulus was 2 kHz. Received level descended from 83 dB re 1 μ Pa (upper trace) in 5-dB steps to 73 dB (lower trace). Arrows indicate the positive and negative peak amplitudes used for threshold analysis. Dashed lines indicate equivalent peaks in the different traces.

Table 2. (a) hearing threshold values of a harbor porpoise and statistical results for two frequencies tested with AM stimuli at three different levels of masking noise (m_0 = no masking, m_1 = moderate masking, and m_2 = high masking level); (b) without masking sound present. The threshold values are given with 95% confidence limits, information on significance (* = significant; ** = highly significant), and further statistical information. Significance reflects the relationship between the received level of the acoustic stimuli and the evoked neuronal responses. The data sets included into the threshold analysis were visually selected; the number of data points included in the analysis is given for every frequency and masking condition. Numerator DF are always 1.

(a)

Frequency	Masking level	Lower confidence limit	Threshold	Upper confidence limit	F	Error DF	Significance	Number of values
[kHz]	m_{0-2}	[dB re $1\mu\text{Pa}_{\text{rms}}$]						
2	0	20.0	62.5	68.1	4.76	31	0.037 *	33
2	1	44.3	57.8	62.4	44.78	38	0 **	40
2	2	56.2	62.6	66.6	22.62	29	0 **	31
2.8	0	49.2	60.5	64.6	6.47	29	0.017 *	31
2.8	1	59.0	61.8	64.0	37.8	33	0 **	35
2.8	2	18.3	51.0	59.5	14.13	48	0 **	50

(b)

Frequency	Masking level	Lower confidence limit	Threshold	Upper confidence limit	F	Error DF	Significance	Number of values
[kHz]	m_{0-2}	[dB re $1\mu\text{Pa}_{\text{rms}}$]						
2	0	20.0	62.5	68.1	4.76	31	0.037	33
2.8	0	49.2	60.5	64.6	6.47	29	0.017	31
4	0	60.5	64.7	67.1	26.92	53	0	55
5.6	0	55.0	59.4	63.2	75.28	54	0	56
8	0	49.5	74.5	79.4	6.45	15	0.023	17
11.2	0	64.1	74.7	80.7	6.56	57	0.013	59
16	0	82.3	85.0	87.2	97.63	52	0	54

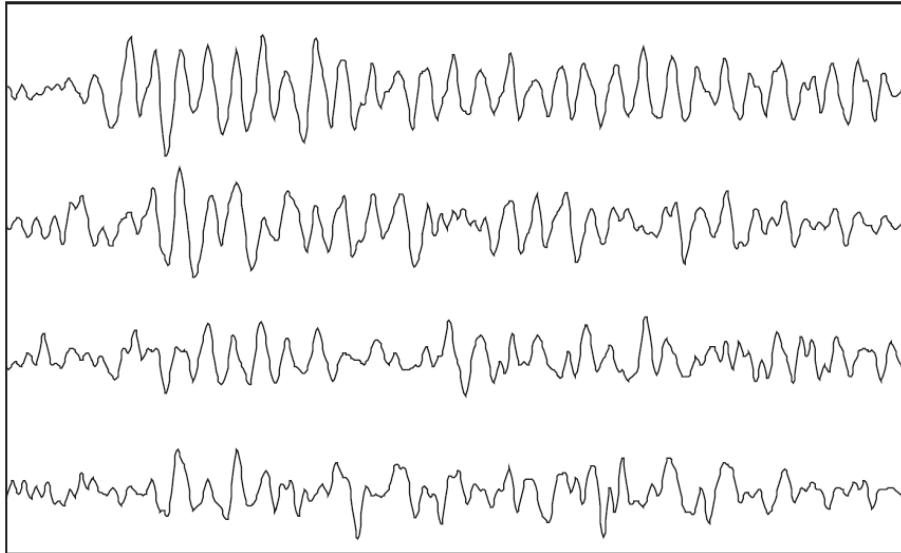


Figure 10. Examples of EFR in a harbor porpoise to AM sound stimuli; sampling duration was 30 ms, carrier frequency was 22.4 kHz, modulation rate was 1.1 kHz and modulation depth was 100%. Received level descended from 76 dB re 1 μ Pa (upper trace) in 3-dB steps to 67 dB re 1 μ Pa (lower trace).

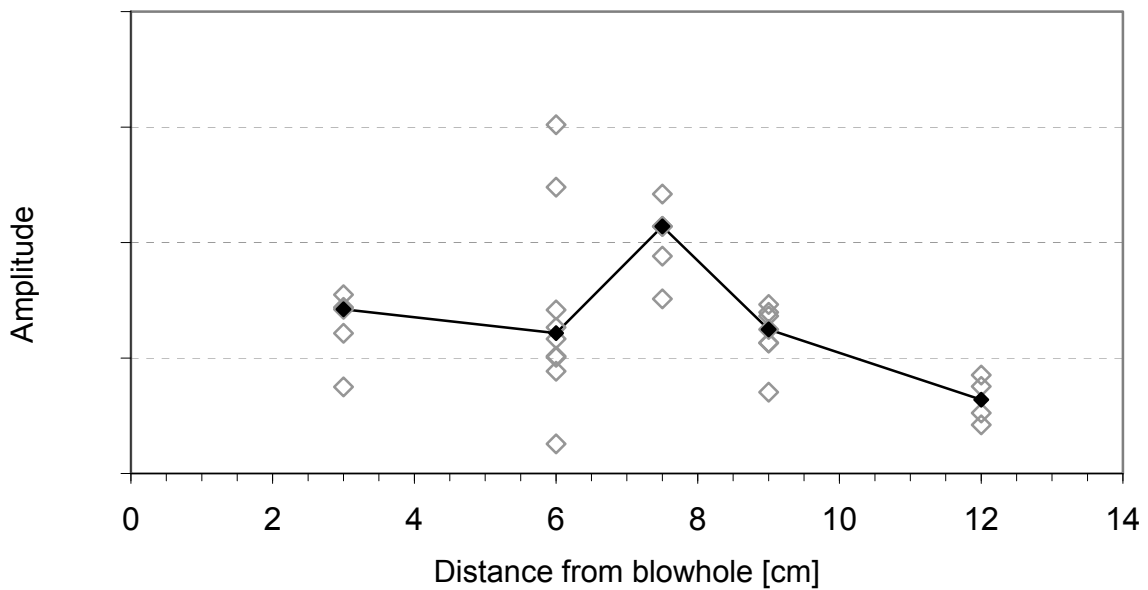


Figure 11. Amplitude dependence of EFR (gray symbols) on the positioning of the active electrode along the longitudinal axis behind the blowhole; AM signals (carrier frequency of 22.4 kHz; modulation frequency of 1.1 kHz) at a received level of 94 dB_{rms} re 1 μ Pa were used as stimuli. The median values are plotted as black symbols.

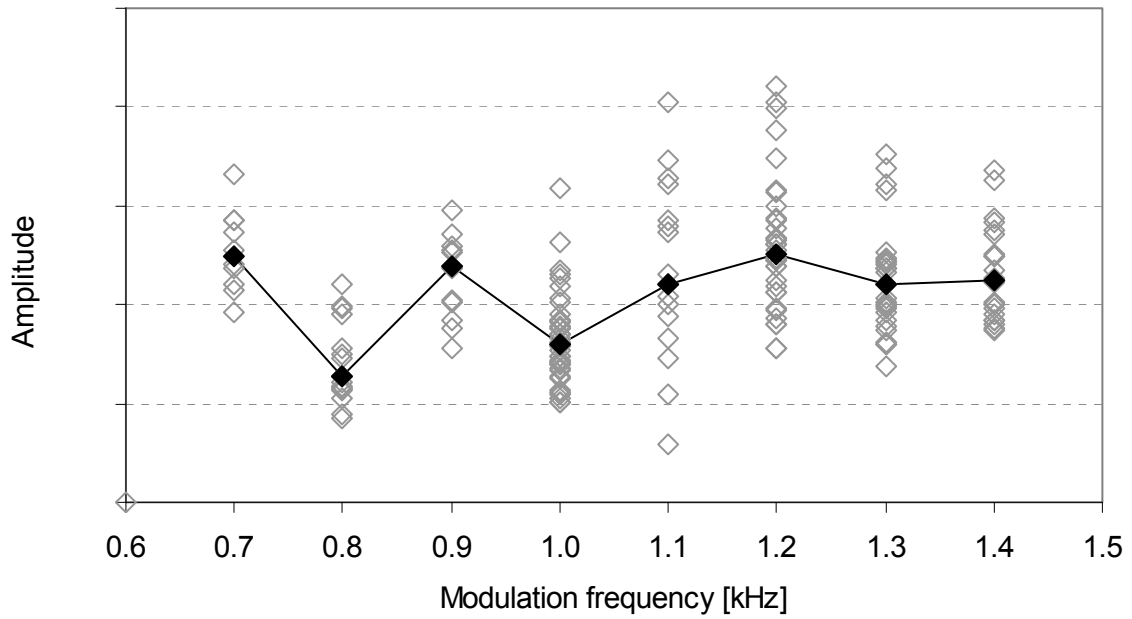


Figure 12. Amplitude of the EFR (gray symbols) as a function of the modulation rate of a carrier signal (carrier frequency of 22.4 kHz; active electrode placed 7.5 cm behind blowhole; received level of 94 dB_{rms} re 1 μPa). The median values are plotted as black symbols.

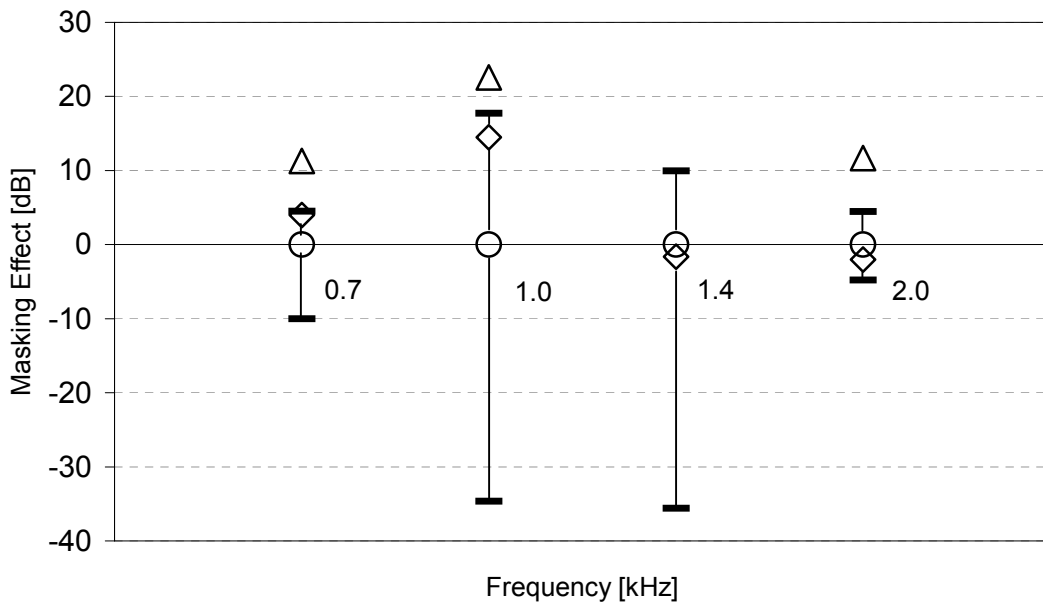


Figure 13. Measured masking effect (in dB) of simulated operational noise of a wind turbine on the hearing threshold of a harbor porpoise at low frequencies; threshold values were achieved by using tone pips at four different frequencies. Circles represent the (relative) hearing threshold with no masking noise present; error bars indicate the 95% confidence intervals. Diamonds show threshold values at moderate masking levels, Triangles represent the threshold values at high levels of masking noise both are relative to unmasked thresholds.

A comparison of the resulting thresholds with comparable data published from other auditory studies on harbor porpoises are given in figure 11¹. Between 2.0 and 5.6 kHz, the resulting sensitivity is in good accordance with other audiometric data published for harbor porpoises so far. In relation to the audiogram measured by Kastelein *et al.* (2002) on the same animal using a behavioral technique, the ABR data revealed even higher sensitivities. At frequencies of 8 kHz and above, the threshold rose – that is, the animal seems to be less sensitive.

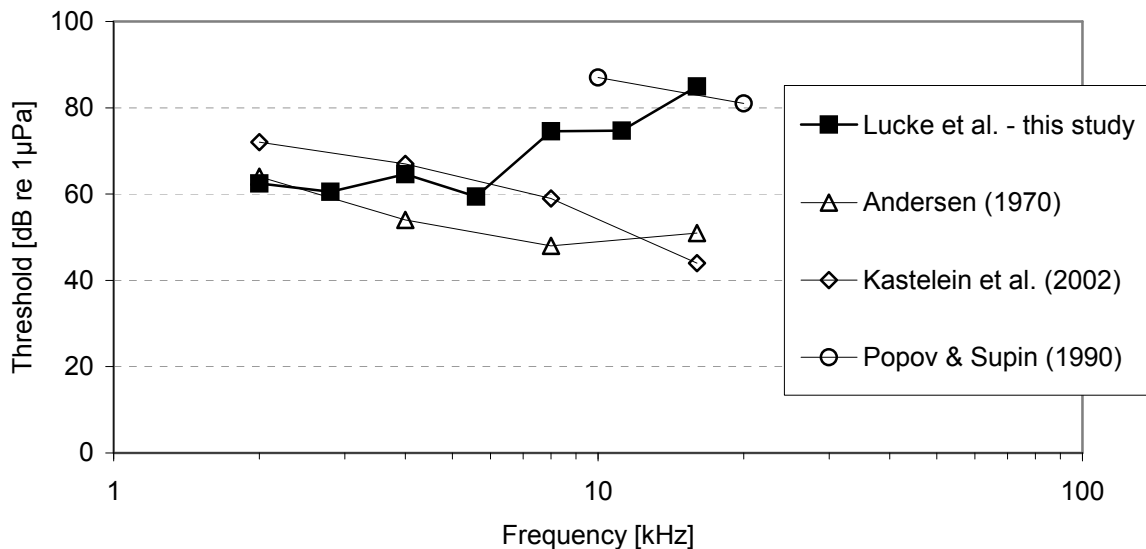


Figure 14. Harbor porpoise hearing threshold data from different studies; the filled symbols represent the threshold values achieved in this study. Data from another AEP study (Popov and Supin, 1990) as well as from two behavioral auditory studies (Andersen, 1970; Kastelein *et al.*, 2002) are given for comparison.

DISCUSSION

Two different types of acoustic stimuli were used in this electrophysiological study to evoke neuronal responses and acquire auditory data from a harbor porpoise. This technique has been successfully used before (Supin *et al.*, 2001, for review) and provides in principle the opportunity to collect data in a relatively short period of time. The acoustic conditions of the research environment in which this study was conducted demanded an intermediate approach, which included behavioral training and active participation of the study animal. The advantage of this was the possibility to conduct tests at low frequencies, the main focus of this study. By having to conduct the AEP measurements on a free-moving animal, however, the signal-to-noise ratio and hence the quality of the resulting AEP data was likely to be compromised.

¹ **Erratum** (does not appear in published version of this manuscript): The comparison of the resulting thresholds with data from other auditory studies is shown in figure 14.

The intensity and acoustic characteristics of sounds associated with offshore wind turbines may vary due to the wind speed, type of wind turbine, bottom substrate, water depth and weather as well as oceanographic conditions and other minor parameters (e.g., direction to the wind turbine at which a signal is perceived). Based on the information gathered from recordings made at several wind farms so far (see Wahlberg and Westerberg, 2005; Madsen *et al.*, 2006, for reviews), the simulated operational sound of an offshore wind turbine used in this study matched those real sound emissions in general. It contained white noise filtered to reduce frequencies above 1.0 kHz with the addition of strong tonal components resembling the major acoustic emissions of the wind turbines. Because there is some variation in the reported noise levels from operating wind turbines, ranging to source levels of up to a maximum of 145 dB_{rms} re 1 µPa (back-calculated from reported measurements; Wahlberg and Westerberg, 2005) for the low frequency tonal components, the masking level was chosen to be as high as possible. The maximum received level of 128 dB_{rms} re 1 µPa should be clearly audible to the harbor porpoise whose hearing threshold was previously measured to be 115 dB_{rms} re 1 µPa at 0.25 kHz and 92 dB_{rms} re 1 µPa at 0.5 kHz (i.e., at or near the frequencies of the tonal components of the simulated sound) (Kastelein *et al.*, 2002).

As revealed by the threshold analysis at the low frequencies (0.7 to 2.8 kHz), this sound had a masking effect on the perception of auditory stimuli by the harbor porpoise. Such a masking effect has been identified from the experiments with tone pips as acoustic stimuli at 0.7, 1.0 and 2.0 kHz. While these data could not be analyzed for the 1.4 kHz data sets, the results from the masking experiment with AM signals as acoustic stimuli revealed no masking effect at 2.0 and 2.8 kHz. Masking occurs if the threshold value analyzed for a moderate or high level of masking noise is above the upper limit of the confidence interval of the threshold value achieved at the frequency in an unmasked condition. Thus, masking occurred at levels between 4.8 dB (at 1.0 kHz) and 7.3 dB (2 kHz), with an intermediate masking effect of 6.8 dB at 0.7 kHz.

The observed variation in threshold values at different levels of masking, as well as the comparatively large confidence intervals can be attributed to the reduced signal-to-noise ratio of the measured AEP signals in this study. Since the animal was unrestrained during the measurements and had to actively position itself constantly in front of its station, the resulting myogenic potentials are likely to have raised the level of the overall neuronal potentials. This elevated level might have especially masked the auditory potentials, which were measured at received acoustic levels near the auditory threshold. This electrophysiological masking effect leads to a higher degree of variation in the analysis of the auditory thresholds as the near-threshold AEPs are influencing the definition of this threshold. In addition, the amplitude of the EFR has been negatively influenced by a reduction in the modulation depth of the AM stimuli due to interference from reflections in the pool. Even though the research conditions were kept as constant as possible, this effect occurred at varying levels at different frequencies and could not be eliminated by the baffles. Moreover, it was also likely to be influenced by other external factors (e.g., water level and wave action). Nevertheless, the observed difficulties in defining the hearing threshold were compensated by means of a complex statistical analysis.

It has to be taken into consideration that the acoustic stimuli used during this part of the experiment was broadband with a spectrum ranging up to a maximum 8.5 kHz with an energy of 5 dB below the maximum value. This acoustic artefact occurred due to the response characteristics of the transmitting hydrophone in relation to the stimulus. Taking into account that the threshold levels achieved with the tone pips as stimuli cannot be regarded as absolute hearing thresholds, the data clearly showed a masking effect. Due to the broadband spectrum, the test stimuli have certainly acoustically stimulated higher-frequency bands on the basilar membrane, rather than being limited to the anticipated frequency bands. At those higher frequencies, the auditory sensitivity should be higher, thus making a perception even more likely. With increasing frequency, those higher-frequency bands should be increasingly less affected by the masking sounds. Thus, with regard to the observed masking, the frequency spread indicated that the actual masking effect of the wind turbine-related sounds could be larger at the frequencies targeted initially. Hence, a narrower test signal would be very likely to reveal a more pronounced masking effect.

If the received level of the operational sounds on average dropped below 120 dB within a range of 100 m from a wind turbine (Madsen *et al.*, 2006), the level of the masking sound used in this study (high masking level: 128 dB re 1 μ Pa) would have been received only at a short distance from an average type of offshore wind turbine (several tens of meters). The difference between the effective masking intensity at the high masking level and the non-effective moderate masking level was approximately 13 dB. Thus, the effective range of the observed masking would be comparatively small as the operational sound of an offshore wind turbine would be attenuated by 13 dB in shallow water within 20 m from the sound source (assuming spreading with a loss of $10 \log r$ [r = distance in m]) and at less than 10 m distance from the sound source in deep waters (assuming spherical spreading with a loss of $20 \log r$). Due to oceanographic or geological features, the spreading loss can reach even higher levels, thus decreasing the effective masking range of the wind turbine sounds. The actual sound measurements have been carried out at comparatively small wind turbines, however. Several offshore wind farms are currently planned to consist of turbines of up to 5 MW. It is unclear to what extent the sound emissions of these turbines will be elevated with the increased turbine size. So far, these emissions have only been modelled (DEWI, 2004) but should be measured upon construction of the turbines.

Nevertheless, with regard to their regulatory implications, these results indicated that the masking effect of the operational sound emissions of wind turbines is small. The perception of sounds which might be biologically meaningful to the harbor porpoises would be constantly reduced, but the masking range is limited to several tens of meters around the wind turbine (i.e., the harbor porpoise will encounter an impaired perception of low- to mid-frequency signals only in the vicinity of the wind turbines). As the distance between turbines will be several hundred meters, the potential masking zone of neighboring wind turbines will not overlap. Therefore, from an ecological point-of-view, the results indicated that the overall effect of the operational sound emissions of the planned wind turbines is very likely to be small for the individual harbor porpoise and negligible on a population level.

Studies on the behavioral reactions of harbor porpoises to continuous sound from offshore wind turbines (Koschinski *et al.*, 2003) indicated that displacement occurs over slightly longer distances than the masking effect. As the overall noise regime in this study differed markedly from the North Sea noise conditions, and due to context-specific variation and the strong individual differences in reactions of the animals, it remains difficult to assess the effective range of behavioral effects from wind turbine sound as compared to the masking.

The conclusions that can be drawn from this study are naturally limited by the sample size of $n = 1$. The normal individual variation in hearing sensitivity could theoretically account for a higher auditory sensitivity of several dB. The audiometric data published for harbor porpoises so far, as well as the results from this study, indicate that at least at the lower frequencies (<5.6 kHz), this variation is confined to 13 dB, whereas the thresholds measured in this study are at low or intermediate levels within this range. Age-related hearing loss (e.g., Moore and Finneran, 2004) that could reduce the masking effect of wind turbine-related sound can be ruled out for this study since at these low frequencies the animal's measured hearing threshold is in good accordance with the levels measured on the same animal when it was young. Moreover, in other odontocetes, age-related hearing loss occurred at much higher frequencies.

The echolocation clicks of harbor porpoises are short, transient signals with a high acoustic energy at frequencies centered around 130 kHz. Apart from these clicks, no other sounds have been recorded so far which can be attributed to serve for communication purposes for these animals. In this context, it has been hypothesized that the low-frequency portion of the click, which has been documented by Verboom and Kastelein (1995) could code some information for the harbor porpoise (Schevill *et al.*, 1969). Within the scope of this study, a low-frequency component could not be documented for either of the two harbor porpoises in the research pool. This does not imply that the low-frequency component does not exist in the porpoise click. The low-frequency component was characterized to have a source level of approximately 100 dB re 1 μ Pa and a frequency spectrum of 1.4 to 2.5 kHz. The range of such a quiet signal is limited by the natural background noise in the sea anyway. Any additional sound source in a similar or adjacent frequency range would be likely to mask the perception of these sounds and, thus, reduce the effective range of this low-frequency component. As the masking effect has been documented for a broadband test stimulus, a masking effect is even more likely to occur for this low-frequency part of the echolocation signal based on the existing data.

The threshold results achieved with AM signals can be considered as absolute hearing threshold values at frequencies between 2.0 and 5.6 kHz. At these frequencies, the resulting sensitivity is in good accordance with other audiometric data published for harbor porpoises so far. In relation to the audiogram measured by Kastelein *et al.* (2002) on the same animal using a behavioral technique, the AEP data revealed even lower threshold values. At frequencies of 8.0 kHz and above, however, the threshold seems to be less sensitive. At these higher frequencies, the confidence intervals for the threshold values are comparatively small, thus indicating a relatively high declarative strength of the data. The rise in threshold

can either be attributed to the fact that *Daan* lost some of its auditory sensitivity at these frequencies over the last five years or the data reflect the presence of ambient masking noise which is unrelated to this study.

The system set-up was designed to enhance the acoustic situation as much as possible. An important factor for a masking study is the existing background noise in the research environment. In the research pool, the noise floor seemed to be relatively low at relevant frequencies. Looking at the $\frac{1}{3}$ -oct analysis of the background noise, however, it is not surprising to see a rise in the energy content with increasing frequency as the effective range of each $\frac{1}{3}$ -oct interval becomes wider with increasing frequency. Given the assumption that the ear of marine mammals can be modelled as a series of bandpass filters, it strongly depends on the width of these filters as to how much the background noise influences the perception of acoustic stimuli. If the animal's auditory filters were also a $\frac{1}{3}$ -oct bandwidth, the sound energy integrated in these filters would also rise with increasing frequency for a uniformly distributed noise floor. In the open ocean, the background noise drops from high levels at low frequencies with increasing frequency to very low levels, and it only rises up again to substantial levels at frequencies above the frequency spectrum of the harbor porpoises (Wenz, 1962). The background noise recorded in the research under relatively undisturbed conditions nevertheless includes a considerable amount of acoustic energy even at higher frequencies so that at frequencies above 10.0 kHz, the $\frac{1}{3}$ -oct level rises to over 90 dB re 1 μ Pa. At higher frequencies (≥ 22.5 kHz), however, the auditory filters of harbor porpoises are not of constant quality (ratio of center frequency of the auditory filter to the passband bandwidth) but of constant bandwidth as shown by Popov *et al.* (2006). Assuming that this auditory property is constant over the animal's entire hearing range, the constant noise floor in the pool might have had some masking effect, but this would not solely explain the increase in *Daan's* hearing threshold at and above 8.0 kHz. As systematic causes for elevated hearing thresholds were attempted to be avoided, they can nevertheless not be ruled-out completely for a study in such an acoustically very challenging environment. The documented difference theoretically also could be attributed to the fact that behavioral audiograms can be affected by the attention of the animal and/or whether it was trained to be conservative or liberal in responses. Ultimately, the measured difference in threshold between the behavioral and the electrophysiological methods on the same animal in the same pool could reflect an actual hearing loss.

Absolute hearing thresholds are basic for predicting the perception of acoustic stimuli, but they do not provide sufficient information on whether a signal will be masked or not. Over the last decades, the amount of sound emitted into the water has increased substantially (Hildebrand, 2004). In this context, it seems necessary to collect more information on masking thresholds and masking-related parameters. Numerous research activities have already focused on the potential effects of sounds on the marine environment; this study is one of them.

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TEMPORARY SHIFT IN MASKED HEARING THRESHOLDS IN A HARBOR PORPOISE (*PHOCOENA PHOCOENA*) AFTER EXPOSURE TO SEISMIC AIRGUN STIMULI

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ABSTRACT

An auditory study was conducted to derive data on temporary threshold shift (TTS) induced by single impulses. This information should serve as basis for the definition of noise exposure criteria for harbor porpoises. The measurements of TTS were conducted on a harbor porpoise by measuring the auditory evoked potentials in response to amplitude-modulated sounds. After obtaining baseline hearing data the animal was exposed to single airgun stimuli at increasing levels. Immediately after each exposure the animal's hearing threshold was tested for significant changes. The received levels of the airgun impulses were increased until TTS was reached. At 4 kHz the predefined TTS criterion was exceeded at a received sound pressure level of 199.7 dB_{pk-pk} re 1 μPa and a sound exposure level (SEL) of 164.3 dB re 1μPa²·s. The animal consistently showed aversive behavioral reactions at received sound pressure levels above 174 dB_{pk-pk} re 1 μPa or an SEL of 145 dB re 1μPa²·s. Elevated levels of baseline hearing sensitivity indicate a potentially masked acoustic perception. Therefore, the resulting TTS levels should be considered masked temporary threshold shift (MTTS) levels. The MTTS levels are lower than for any other cetacean species tested so far. They could be indicative for the other "high frequency cetaceans", i.e. species with a frequency spectrum of their echolocation signals above 100 kHz.

INTRODUCTION

Anthropogenic sound resulting from shipping, industrial and military activities and many other sources has led to a substantial increase in the underwater background noise in the oceans over the past decades (Hildebrand, 2004). The North and Baltic Seas are among the most intensively used and consequently noisiest marine areas (OSPAR Commission, 2000). Seismic surveys are one of the most prominent contributors to the overall noise budget in these areas, as in almost all oceans. Consequently, these surveys moved into the focus of interest of scientists as well as policy makers due to the intensity of the emitted sounds and spatiotemporal scale of these activities. Seismic surveys are conducted covering vast areas while searching for hydrocarbon deposits – in the central North Sea the most recent campaign was conducted at the Doggerbank area in spring / summer 2007. The total source level of airgun arrays used as sound source during these surveys depends on size, number and timing of the individual airguns. With source levels ranging from 225 to 255 dB re 1 μPa_{pk} (Richardson *et al.*, 1995), seismic surveys are routinely conducted continuously over several weeks, with repetition rates of several signals per minute.

The acoustic emissions produced during these programs may reach intensities with a potential of causing a variety of effects in the marine fauna at considerable distances – from behavioral reactions (McCauley *et al.*, 2000; Tougaard *et al.*, 2003) and potential stress to physiological effects (Finneran *et al.*, 2002), injury (McCauley *et al.*, 2003) and possibly death (Ketten *et al.*, 1993).

Most odontocete species are known to produce, and be sensitive to, sound (see review in Richardson *et al.*, 1995; Wartzok and Ketten, 1999). They are represented in the central and southern North Sea, the Baltic Sea and especially in German waters by the harbor porpoise (*Phocoena phocoena*) as the only resident whale species. Harbor porpoises have a very acute sense of hearing underwater (Andersen, 1970; Kastelein *et al.*, 2002) and have been shown to use echolocation to find their prey (Busnel *et al.*, 1965; Busnel and Dziedzic, 1967) as well as for spatial orientation and navigation underwater (Verfuß *et al.*, 2005). Their acoustic sense has evolved to be their dominant sense vital to their survival. Any impairment or damage to their auditory system would have deleterious consequences for the affected individuals.

Auditory studies on terrestrial animals have shown that the exposure to intense impulsive sounds could exceed the tolerance of their auditory system and lead to an increased hearing threshold (Ahroon *et al.*, 1996; Kryter, 1994; Yost, 2000). Such a noise-induced threshold shift (TS) can either be temporary (TTS) or permanent (PTS), depending on the hearing system's capacity for recovery once the sound has ceased. A similar cause-effect relationship has been found in odontocetes as TTS has been demonstrated in bottlenose dolphins (*Tursiops truncatus*) (Nachtigall *et al.*, 2003, 2004), belugas (*Delphinapterus leucas*) (Schlundt *et al.*, 2000; Finneran *et al.*, 2002) after exposure to intense intermittent or continuous noise. The TTS data obtained so far indicated that the energy flux density (i.e. the acoustic energy over time or Sound Exposure Level, SEL) of a signal can be used in combination with a maximum peak pressure to determine noise exposure criteria for marine

mammals. As SEL is calculated by integrating the squared sound pressure over a standard unit of time, the duration of a signal plays an important role with regard to TTS. It is still unclear whether the dose-response function follows an “equal-energy rule” in marine mammals, but in the absence of specific data it can be used as a first-order approximation, as pointed out by Southall *et al.* (2007).

Based on these TTS data, a peak pressure of 224 dB re 1 μ Pa and an SEL of 183 dB re 1 μ Pa²·s were initially proposed as noise exposure criteria for mid-frequency cetaceans (e.g. bottlenose dolphins and belugas) for exposures to pulsed sound (Ketten and Finneran, 2004). With the noise exposure criteria proposed by Southall *et al.* (2007), the focus of marine mammal policy has shifted towards PTS and the onset of behavioral disruption. They proposed appropriate interim noise exposure criteria for all toothed whale species based on the dose-response functions found in the two cetacean species tested for their TTS limit so far (see above). The relevant PTS level for single impulses is set for all toothed whale species to a peak pressure of 230 dB_{pk} re 1 μ Pa and a SEL of 198 dB re 1 μ Pa²·s. A criterion for SEL has also been set for the first time for multiple exposure to impulsive sounds, which are likely to lead to a reduced tolerance of the auditory system (Ahroon *et al.*, 1996). This threshold (198 dB re 1 μ Pa²·s) is identical to the SEL criteria for single impulses.

The subjects from former TTS studies are categorized as mid-frequency cetaceans with the main energy of their echolocation clicks and their range of best hearing sensitivity <100 kHz. Harbor porpoises, in contrast, are categorized as “high-frequency” cetaceans (Ketten, 2000; Southall *et al.*, 2007), with a best hearing sensitivity at frequencies above 100 kHz (Andersen, 1970; Kastelein *et al.*, 2002) and an energy maximum of their echolocation signals in the range of 110 to 140 kHz (Verboom and Kastelein, 1995). There are no TTS data available for this species, or for any other “high-frequency” cetacean species. These differences in their acoustic and auditory characteristics may also be reflected in differences in the overall tolerance of their auditory systems to intense noise. Accordingly, a transfer of the first order approximated auditory dose-response function to the harbor porpoise could be questionable. The same applies to an application of the noise exposure criteria defined by Southall *et al.* (2007) to assess effects of pile driving impulses on harbor porpoises (as generated e.g. during the construction of wind turbines).

To base the assessment of acoustic effects of impulsive noise on species-specific data, a dedicated TTS study was conducted on one harbor porpoise. A key element for the planned study was access to a harbor porpoise trained to participate in experiments so that the experiments could be conducted under controlled conditions and definitive information on the dose-response function gathered. The aim of this acoustic study was to define the tolerance limit of the auditory system of the harbor porpoise to single impulsive sounds. Such data would enable regulatory agencies to define “zones of impact” (Richardson *et al.*, 1995) around the construction sites. At the same time, such data could be applied as a more robust baseline in the definition of noise exposure criteria for other “high-frequency” cetacean species (see outline by Southall *et al.*, 2007).

II. METHODS

A. SUBJECT AND FACILITY

A male harbor porpoise held under human care in the Fjord & Bælt Centre (F&B) in Kerteminde, Denmark, was chosen as subject for the studies. This animal was estimated to be between 9 and 10 years old, with a length of 143 cm and an average weight of 40 kg in 2005 when the study began. A comprehensive medical record of all treatments exists for *Eigil* for almost his entire life. He was held in this facility with two female harbor porpoises at that time. The older female was pregnant twice during the study period from 2005 until 2007 and gave birth to a female calf right after the end of the studies in summer 2007. The design of the auditory experiments was altered due to the pregnancies and thus they are relevant for discussion of the results.

The animals were held together at the F&B in a semi-natural outdoor pool of 30 m by 20 m and an average depth of 4 m. Their enclosure stretches along the entrance from the Baltic Sea to a small fjord on one side of the busy fishing harbor of Kerteminde. It has a natural sea bottom and solid walls of concrete and steel on the two long sides. It is separated from the harbor on its narrow ends by nets, thereby providing a constant water exchange with the Baltic Sea. The enclosure is divided into two compartments ('main pool' and 'research pool'), allowing separation of the animals for experiments. A floating pen (4.5 m x 4.5 m x 1.5 m) in the research pool was wrapped with sound absorbing foam, providing an acoustic shelter for the two females during the planned exposures to intense sound in the later stage of the study.

All experiments were conducted with *Eigil*, who was separated temporarily from the two females to avoid behavioral or acoustic interference between the animals during the research. *Eigil* was trained to accept the electrodes that were attached to his head and back with suction cups and to dive on command to an underwater station at 1.5 m water depth. The training method used was based on operant conditioning and positive reinforcement (Pryor, 1984; Ramirez, 1999). No food deprivation was used during these experiments. He stationed himself actively at the set-up with his rostrum touching a 4 cm x 4 cm PVC plate in front of the sound transducers (figure 1) for the hearing tests.

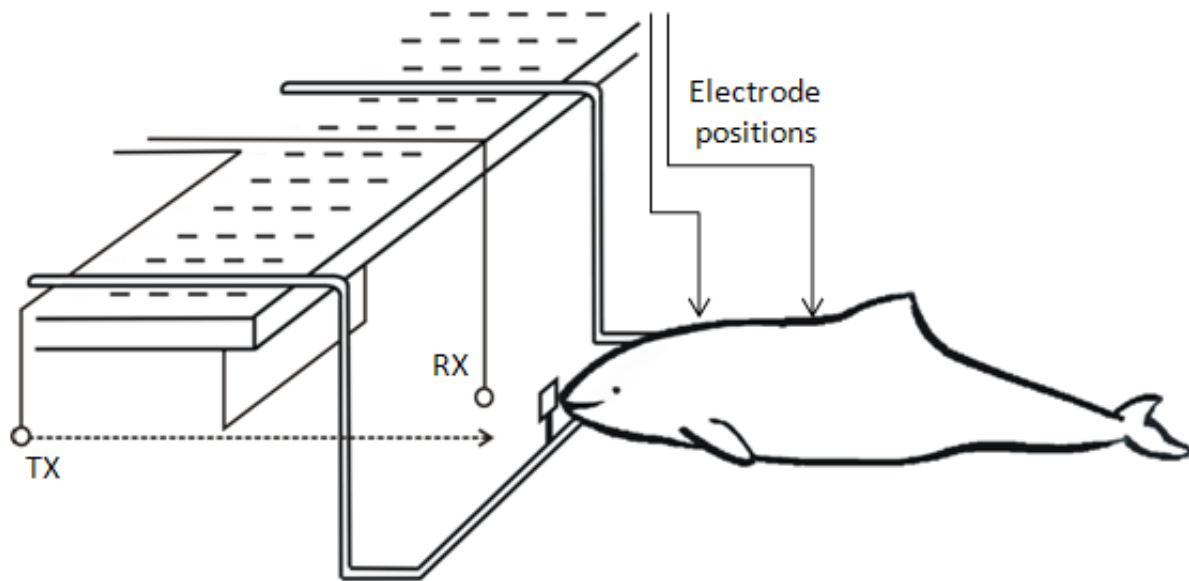


Figure 1. Schematic plot of the research setup for the AEP measurements with the animal positioning itself at 1.5 m water depth in front of its underwater station and with its body in a straight line with the sound path of the incoming AEP stimuli. The direct sound path is indicated by a dashed line between sound source (TX) and monitoring hydrophone (RX) and the animal's position. The animal was held at the F&B in a semi-natural outdoor pool of 30 m by 20 m and an average depth of 4 m.

He stayed there for 100 s on average until he was called back to the surface by the trainer to receive a reinforcement. This experimental sequence was called a 'send'. A complete research session was comprised of four sends on average. The number of research sessions per day depended on weather conditions and varied between 1 and 4 during the study period with an average of 2 sessions, ideally one in the morning and one in the afternoon.

B. STUDY DESIGN

The study was divided into two modules: The first consisted of measurements of the animal's absolute hearing thresholds over almost its entire functional frequency spectrum, thus providing a baseline for the second module, a tolerance test (TTS test) of the animal's hearing. This TTS test followed the same procedural structure as the experiments conducted by Finneran *et al.* (2002). The animal's hearing thresholds were measured in half octave steps over 5.5 octaves with the lower frequency limit set by the methodological parameters of the AEP stimulation. The threshold measurements were repeated several times at three selected frequencies (representing the low, mid and high frequencies of its functional hearing range) to measure normal variation. This would subsequently allow definition of a frequency-specific TTS criterion.

C. MEASUREMENT OF AUDITORY SENSITIVITY (AEP METHOD)

The measurement of auditory evoked potentials (AEP method) was chosen to measure the hearing thresholds in the harbor porpoise as it allows a comparatively rapid data acquisition and is non-invasive. For this reason the technique has been widely adopted in human patients and is also used for screening newborns (Hall, 2006). This technique is based on the presentation of acoustic stimuli which will generate neuronal potentials in the acoustic system upon perception of these stimuli (Picton, 1987). Two surface electrodes are placed on the animal's skin using suction cups – one near the blowhole and the other near the dorsal fin – to record the neural responses evoked within the auditory system (Supin *et al.*, 2001). These potentials are generated within neuronal nuclei at different positions in the auditory system, thereby forming an electric field which can be detected and recorded even on the skin surface. AEPs are useful for measuring the functioning of the auditory system and examining important aspects of auditory processing. To distinguish these comparatively small electric potentials from the overall neuronal activity – i.e. electric activity of the animal's musculature, other sensory inputs, etc. – the acoustic test stimuli are presented at a high repetition rate. By coherently averaging the evoked potentials (e.g. more than 500 AEPs), non-acoustic neuronal signals and incoherent acoustic signals not associated with the acoustic stimuli are reduced or eliminated.

A refined methodological approach is based on the use of rhythmic sound modulations. By sinusoidally modulating the amplitude of carrier tone or sound pulse sequence, it is possible to elicit a neuronal response which includes a specific frequency component correlated to the modulation frequency used. This effect occurs because the auditory system is capable of following the envelope of a sinusoidal signal and producing corresponding neuronal potentials, called envelope-following response (EFR). By applying a fast-Fourier transformation (FFT) analysis, the modulation frequency component can be identified and quantified. The resulting amplitude of the EFR represents the energy content of the neuronal response at the given modulation frequency. The strength of this EFR response can simultaneously be taken as a relative measure for the perception of the carrier frequency of the amplitude-modulated (AM) signal. At each frequency, the stimuli were presented in decreasing intensity, starting at a clearly audible level, until a (neuronal) response was no longer detected. The resulting data were statistically tested for significance by using an F-test to identify EFR responses from arbitrarily occurring noise at the given AM frequency (Finneran *et al.*, 2007).

D. SOUND GENERATION AND DATA ACQUISITION

The animal's hearing was tested at frequencies between 4 kHz and 160 kHz with sinusoidally amplitude-modulated signals (modulation rate: 1.2 kHz) as AEP stimuli. The signals were of 25 ms duration with a modulation depth of factor 1. A custom-made software application was used to program all acoustic stimuli transmitted to elicit the AEPs during the hearing threshold tests. The signal generation system consisted of a data acquisition card (National Instruments DAQ 6062 E) and two function generators (Thurlby Thandar TG 230 and Agilent 33220A – with the first triggering the latter). At frequencies between 4 kHz and 8 kHz all signals were amplified by a power amplifier PA 100E (Ling Dynamic Systems Ltd., Royston, UK). At higher frequencies a power amplifier Brüel & Kjaer 2713 was used to amplify the signals. Due to differences in their transmit response and the geometry of the pool, five different sound transducers had to be used to transmit the acoustic stimuli during the AEP tests: Signals at 4 kHz and 8 kHz were transmitted via an underwater transducer USRD J-9, at 16 kHz and 80 kHz via a Reson TC 4033, at 22.4 kHz via a SRD 4" ball hydrophone, at 44.8 kHz via a SRD HS70 and all remaining frequencies were transmitted via a SRD HS150 hydrophone. All transmitted and received signals were constantly observed in real time at an oscilloscope and recorded for post-analysis via a monitoring hydrophone (Reson TC 4014) and a preamplifier (Etec B1501) for received level, signal quality and undesired signal artefacts using software packages SeaProDaq (Pavan *et al.*, 2001) and custom software LU-Daq. The evoked potentials were fed into a custom-built input station consisting of an amplifier (20 dB gain) and an optical separation unit (including 20 dB gain). Additionally, the signals were band-pass filtered (high-pass frequency: 300 Hz, low-pass frequency: 10 kHz, NF Electronic Instruments FV-665) to avoid artefacts. Each sequence of 500 successive potentials was averaged and displayed online as well as stored for post-hoc analysis.

The background noise in Kerteminde harbor is dominated by shipping noise from a variety of boat traffic ranging from recreational and small fishing boats passing the enclosure to fishing boats turning into the unloading area on the opposite side of the harbor and supply vessels for a nearby island. The background noise was thus dominated by low frequency noise at varying levels and frequencies, depending on the size, speed and activity of the respective boats.

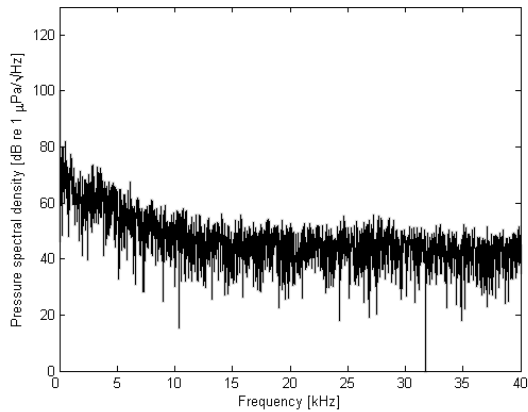


Figure 2. Background noise level (plotted as pressure spectral density) recorded in the research pool at F&B during quiet conditions.

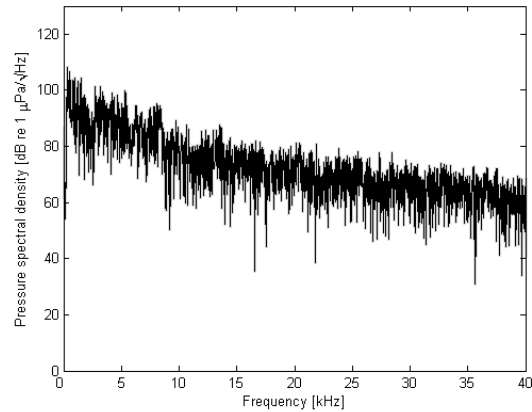


Figure 3. Background noise level (plotted as pressure spectral density) recorded in the research pool at F&B recorded at the same position as in figure 2 during noisy conditions (motorboat turning at 50 m distance).

E. SOUND EXPOSURE PROCEDURE

A threshold shift (TS) was defined as a difference of twice the standard deviation from the average hearing threshold at the particular frequency applied. The TTS criterion of 6 dB as proposed by Southall et al. (2007) was used as a second, frequency-independent criterion in this study. The tolerance of the animal's auditory system was then tested by first exposing the animal to a sound impulse as a fatiguing stimulus and then immediately re-measuring the hearing threshold. Any reduction in the animal's hearing sensitivity exceeding the preset TTS criterion would be regarded as evidence of an actual threshold shift. Subsequent measurements of the animal's hearing threshold at the affected frequency would provide information about the recovery function of the auditory system.

All three frequencies were tested separately for TTS at a given exposure level of the fatiguing stimulus; i.e. only one hearing frequency was tested after each exposure. As long as the hearing threshold was shown to remain within its normal variation at all three frequencies, the subsequent exposure level of the fatiguing stimulus would be elevated and this procedure repeated until a threshold shift is detected. This precautionary approach was chosen to avoid any risk of permanent hearing loss.

Various metrics have been used for both peak and energy amplitude, hearing threshold, spectral level and spectral density, many discussed by Madsen (2005). A summary of calculation methodology is given below. Where possible, reported units are provided in formats used in other relevant studies to allow comparison with previous results.

For a specific pulse, the peak-to-peak pressure (P_{pk-pk}) is calculated from the pressure, p , by the expression:

$$P_{pk-pk} = \max(p) - \min(p)$$

where $\max(p)$ and $\min(p)$ are the peak positive and peak negative pressures in the waveform, respectively. Since the peak may have a negative pressure, the peak-to-peak pressure is equivalent to the sum of the magnitudes of the peak positive and peak negative pressures. Peak pressure is defined as the maximum magnitude of peak positive or peak negative pressure. The value is expressed as the peak-to-peak sound pressure level in dB re 1 μ Pa. This is calculated from:

$$SPL_{pk-pk} = 20 \log \left[\frac{P_{pk-pk}}{P_0} \right]$$

where P_0 is the reference pressure of 1 μ Pa (peak-to-peak).

The SEL for a single pulse is the integral of the square of the pressure waveform over the duration of the pulse using a 90 % energy criterion. The duration of the pulse is defined as the region of the waveform containing the central 90 % of the energy of the pulse. Given by:

$$E_{90} = \int_{t_5}^{t_{95}} p^2(t) dt$$

The value is then expressed in dB re 1 μ Pa²·s and is calculated from:

$$SEL = 10 \log \left[\frac{E_{90}}{E_0} \right]$$

where E_0 is the reference value of 1 μ Pa²·s, t_5 is the time of a 5 % increase in energy for the total pulse energy and t_{95} the time of 95 % of the total energy of the pulse. The pulse duration is therefore defined as the time taken from 5 % to 95 % of the total pulse energy.

The root mean square (rms) pressure was calculated by taking the square root of the average of the square of the pressure waveform over the duration of the pulse, again using a 90 % energy criteria, with the pulse duration defined as above. This is given as:

$$P_{rms} = \sqrt{\frac{1}{T_{90}} \int_{t_5}^{t_{95}} P^2(t) dt}$$

The value was expressed as the rms sound pressure level in dB re 1 μ Pa (rms). This is calculated from:

$$SPL_{rms} = 20 \log \left[\frac{P_{rms}}{P_0} \right]$$

where P_0 is the reference pressure of 1 μ Pa (rms).

A small sleeve airgun (20 in³) was used as sound source to produce the fatiguing sound stimuli during the second module. This device was pressurized with nitrogen at a pressure of 137 bar (2000 PSI) and was operated at a depth of 2 m (i.e. in mid-water) from a small inflatable boat (source boat) in Kerteminde harbor at varying positions between the F&B and the eastern exit of the harbor area. The exact position of the source boat was determined by GPS, and this information, along with time, weather conditions and other relevant information on the sound source, was documented for further analysis. An intensive calibration of the airgun had been conducted prior to the study to predict the received levels of the airgun stimuli as a function of its distance to the receiving position in the main pool at the F&B.

The sudden release of pressure from the airgun during a ‘shot’ results in an oscillating air bubble which projects a short, intense impulse (figure 4) into the water and across adjacent boundaries (ground wave; audibility of airgun shot in air). The main acoustic energy of this impulse is centered below 500 Hz but considerable energy can also be detected up to above 20 kHz, well above background noise in quieter periods (figure 5).

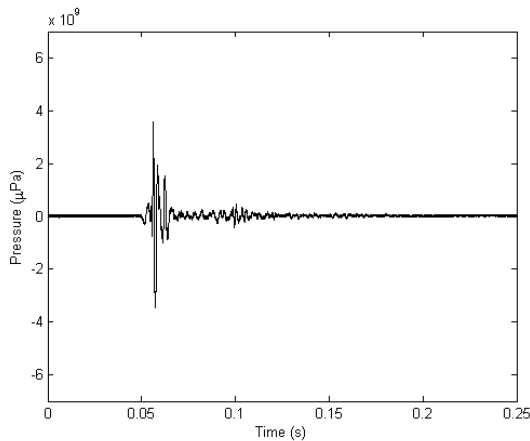


Figure 4. Time domain representation of an airgun impulse. The airgun was fired at 2 m water depth in Kerteminde harbor and the impulse was recorded at a distance of 15 m to the receiving hydrophone.

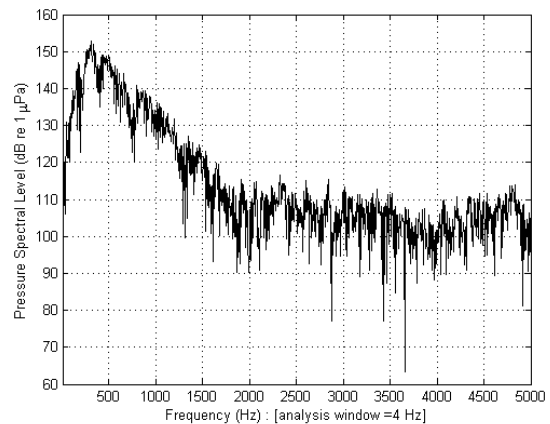


Figure 5. Frequency spectrum analysis of the recorded airgun impulse (figure 4) showing the pressure spectral level [dB re 1 μ Pa]. The frequency spectrum is plotted in Hz, the spectrum levels are based on a 4 Hz analysis band.

Prior to each airgun shot, the two female harbor porpoises were separated into the sound-insulated floating pen. Their general behavior and breathing rates were observed for the period of the sound exposure and compared with baseline data previously obtained under normal conditions. *Eigil* remained in the main pool. A receiving hydrophone was positioned at 1.5 m water depth at a position at the narrow end of the pool facing the eastern exit of Kerteminde harbor. This position had proven to receive the most intense signals during the airgun calibration. The airgun was triggered as soon as *Eigil* was within approximately 1 m of the receiving hydrophone with his body fully underwater. Control

experiments were repeatedly made by conducting the complete procedure except for the exposure to the fatiguing stimulus. The animal's behavior was monitored and video recorded for further analysis. Immediately after each exposure to the fatiguing stimulus, the animal was then led into the research pool where the AEP setup was located. The post-exposure AEP measurements began less than 4 min after the exposure and typically were concluded within 12 min. Within this period his hearing sensitivity could be determined at a single frequency. During this second module, *Egill's* hearing sensitivity was tested at 4 kHz, 32 kHz and 100 kHz.

III. RESULTS

A. HEARING THRESHOLD

Egill's baseline audiogram was determined at frequencies between 4 kHz and 140 kHz. At the highest frequency tested, 160 kHz, no AEP responses were detected. The measurements of *Egill's* auditory sensitivity at the remaining frequencies resulted in elevated thresholds compared to hearing data published for other harbor porpoises (figure 6).

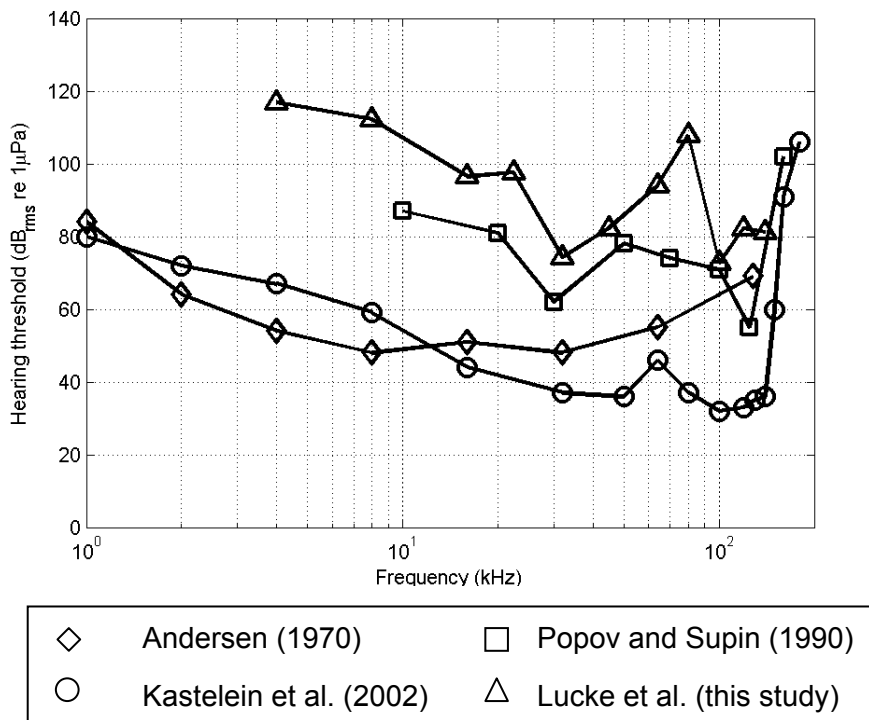


Figure 6. Harbor porpoise hearing threshold data from different studies. The triangles represent the threshold values achieved in this study. Data from another AEP study (Popov and Supin, 1990) as well as from two behavioral auditory studies (Andersen, 1970; Kastelein *et al.*, 2002) are given for comparison.

The shape of *Eigil's* hearing curve with its two minima at the mid and high frequency range is in good accordance with the previously published data. However, a clear rise in threshold was measured compared to data obtained by Kastelein *et al.* (2002) in a behavioral hearing study, with the maximum difference at 80 kHz. At the higher frequencies, the difference is not as pronounced compared to the thresholds obtained by Andersen (1970) but thresholds are still 10 to 20 dB higher. Compared to the results from the AEP study by Popov and Supin (1990), *Eigil's* thresholds are elevated by roughly 10 dB. The mean hearing thresholds at 4 kHz, 32 kHz and 100 kHz respectively, which were chosen as representative frequencies for the low, mid and high range of the animal's functional hearing spectrum, were at 116.9, 74.2 and 72.7 dB_{rms} re 1 μPa. Based on the variation of the hearing thresholds measured during the first module, the TTS criteria were defined as 122.9 dB_{rms} re 1 μPa at 4 kHz, 79.0 dB_{rms} re 1 μPa at 32 kHz and 85.7 dB_{rms} re 1 μPa at 100 kHz.

B. TTS TESTS

Over a period of 4.5 months, *Eigil* was exposed to a total of 24 airgun impulses. The received peak pressure of the pulses ranged from 161.2 dB_{pk-pk} re 1μPa to 202.2 dB_{pk-pk} re 1μPa, with an acoustic energy (SEL) ranging from 140.5 dB re 1μPa²·s to 167.2 dB re 1μPa²·s. These levels were achieved using source ranges of between 150 m and 14 m from the animal's position during the exposure.

1. THRESHOLD SHIFTS

A temporary threshold shift (TTS) was first measured after *Eigil* had been exposed to an airgun impulse at a peak pressure of 200.2 dB_{pk-pk} re 1 μPa with corresponding SEL of 164.5 dB re 1μPa²·s. The threshold shift was measured when the animal hearing was tested after the exposure for its sensitivity at 4 kHz. Since this threshold shift was only 1.8 dB above the predefined TTS criterion, the exposure was repeated a few days later with a received peak pressure level of 202.1 dB_{pk-pk} and a SEL of 165.5 dB re 1 μPa²·s. The resulting threshold shift at 4 kHz was 9.1 dB above the TTS criterion and hence a clear support of TTS. Another verification of this effect was achieved two days later, after an exposure at a peak pressure level of 201.9 dB_{pk-pk} re 1 μPa with a SEL of 165.8 dB re 1 μPa²·s, when *Eigil's* hearing revealed a threshold shift at 4 kHz of 15 dB (figure 7). No significant elevation of hearing threshold at 32kHz was observed at a comparable exposure level to the 4 kHz test case. The received energy was similar to the 4 kHz case, but a slightly lower received peak-to-peak pressure was observed (figure 8). At 100 kHz a change in hearing sensitivity was observed at lower levels (figure 9). The shift in hearing threshold exceeded the 6 dB criterion by more than 2 dB but it did not exceed the frequency-dependent criterion of two standard deviations. This effect did not appear again at higher exposure levels. It should be noted that the airgun source itself creates less energy at the mid and high frequency range than at 4 kHz.

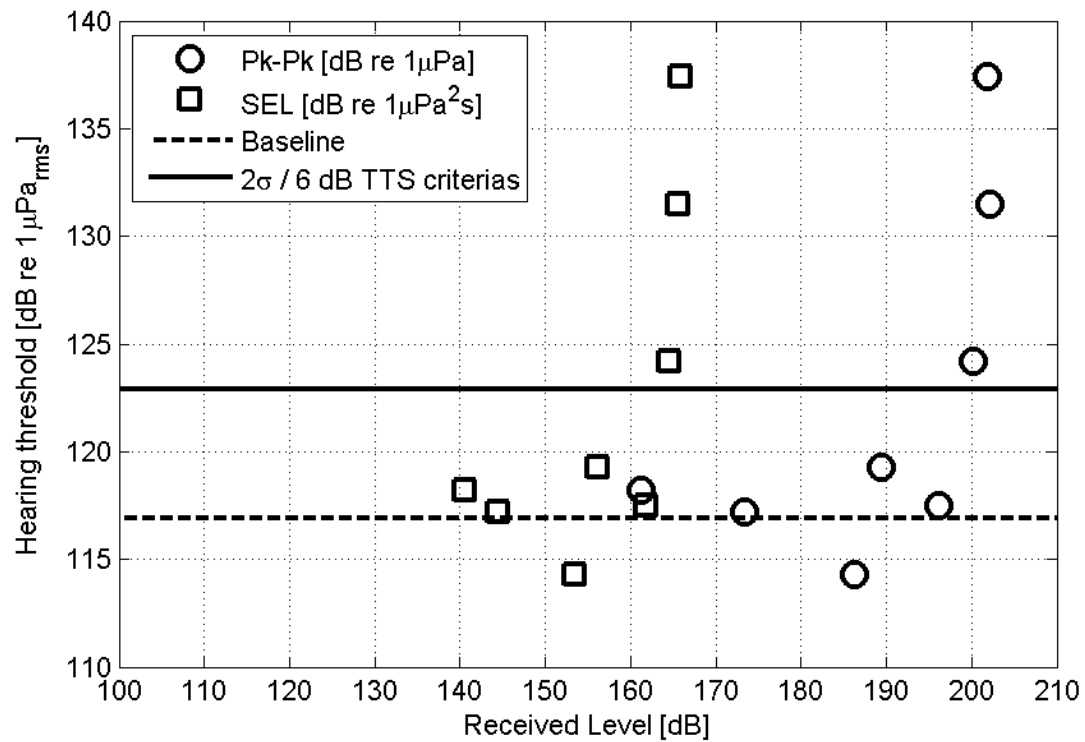


Figure 7. Hearing threshold at 4 kHz for a harbor porpoise after exposure to airgun stimuli (i.e. post-exposure) at different received levels plotted in relation to the animal's pre-exposure hearing sensitivity. Each post-exposure hearing threshold is plotted twice - circles indicating the received the peak-to-peak pressure of the fatiguing stimuli and squares the equivalent received SEL levels of the same exposure impulses. The dashed line represents the normal hearing threshold and the solid line the two TTS criteria used for comparison (which are identical at 4 kHz). Symbols above the solid line indicate a temporary shift of hearing threshold.

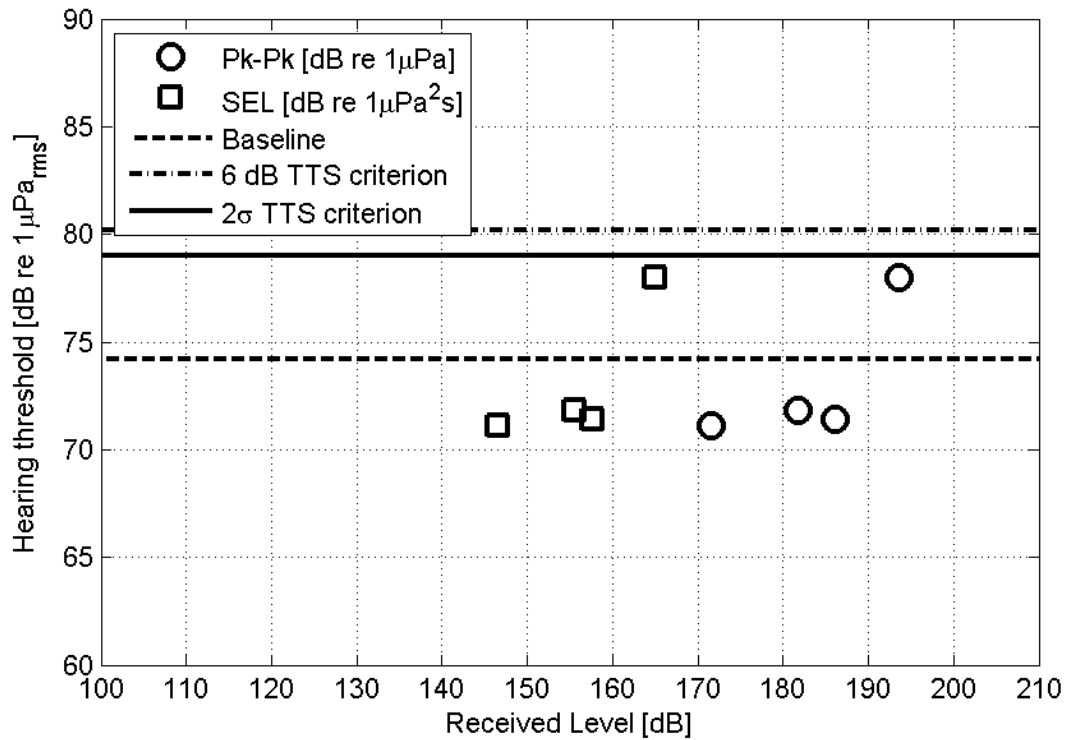


Figure 8. Hearing threshold at 32 kHz for a harbor porpoise after exposure to airgun stimuli (i.e. post-exposure) at different received levels plotted in relation to the animal's pre-exposure hearing sensitivity. Each post-exposure hearing threshold is plotted twice - circles indicating the received the peak-to-peak pressure of the fatiguing stimuli and squares the equivalent received SEL levels of the same exposure impulses. The dashed line represents the normal hearing threshold. The other lines indicate the two different TTS criteria used for comparison.

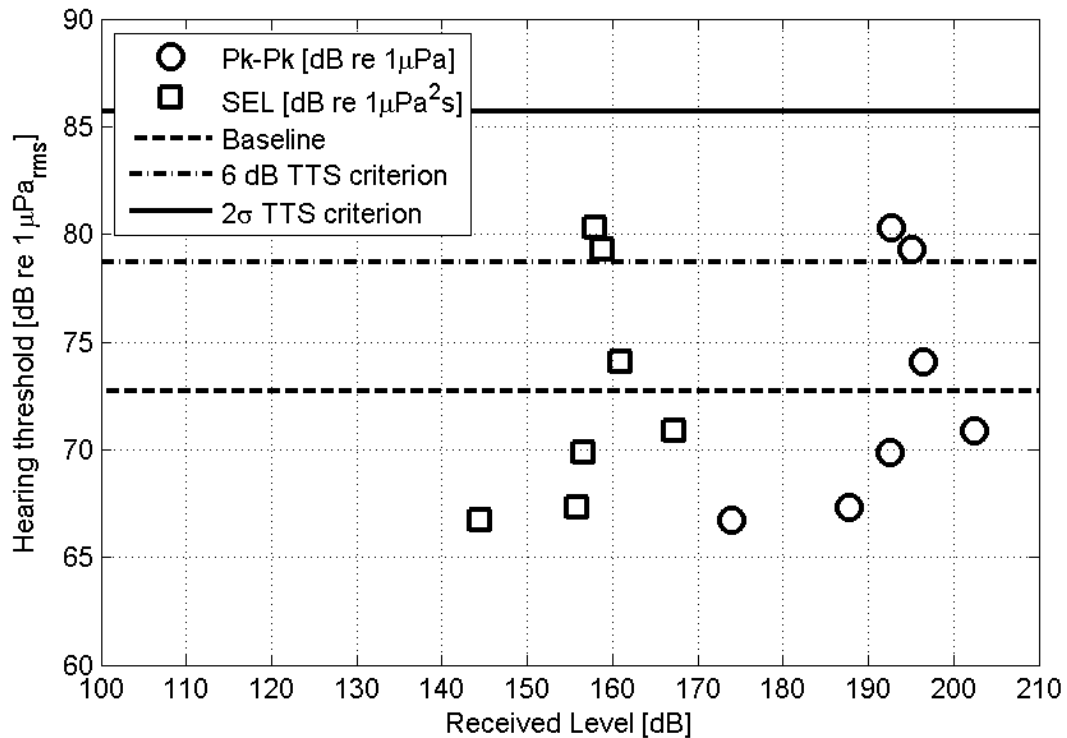


Figure 9. Hearing threshold at 100 kHz for a harbor porpoise after exposure to airgun stimuli (i.e. post-exposure) at different received levels plotted in relation to the animal's pre-exposure hearing sensitivity. Each post-exposure hearing threshold is plotted twice - circles indicating the received the peak-to-peak pressure of the fatiguing stimuli and squares the equivalent received SEL levels of the same exposure impulses. The dashed line represents the normal hearing threshold. The other lines (dot-dashed line and solid line) indicate the two different TTS criteria used for comparison, symbols above one of those lines indicate a temporary shift of hearing threshold.

2. RECOVERY

An important factor for the assessment of this noise-induced effect is the recovery of the animal's auditory system. After the first clear threshold shift had been measured, a series of AEP measurements was conducted over the following days to follow the further development of *Egill's* hearing sensitivity at the affected frequency. 178 minutes after the initial exposure his hearing had recovered only partially from its threshold shift. It was reduced by 2.9 dB but still being elevated above the TTS criterion. *Egill's* sensitivity at 4 kHz improved by 3.5 dB 269 minutes post exposure but only by another 1.4 dB 29 hours post exposure. The same tendency can be identified after TTS was observed in another exposure to the airgun stimulus (figure 10).

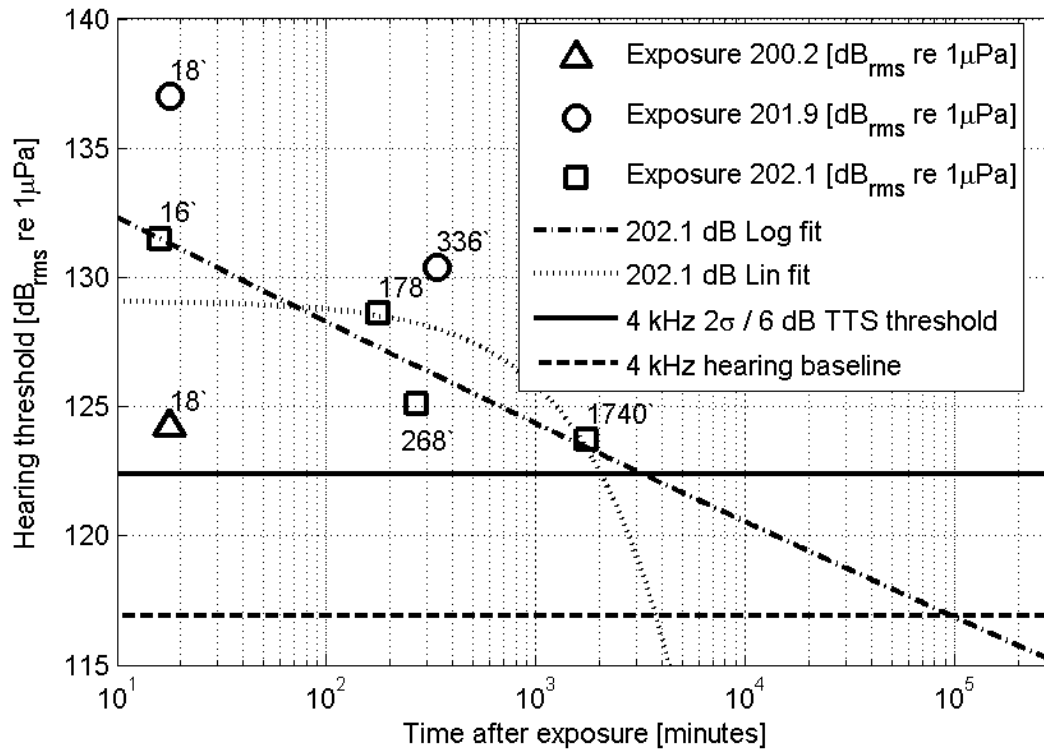


Figure 10. Recovery function of a harbor porpoise's hearing threshold at 4 kHz after sound-induced threshold shifts. Hearing thresholds measured subsequent to the exposures after different times (given in minutes next to the symbols) are indicated by different shapes for every exposure to the fatiguing stimulus. The recovery function for the exposure at 202,1 dB_{rms} re 1 μPa is indicated by the diagonal and curved lines. The dashed line represents the normal hearing threshold and the solid line the two TTS criteria used for comparison (which are identical at 4 kHz).

Assuming a linear recovery from TTS, the animal's hearing sensitivity would have reached the TTS criterion level again in 12 hours for the 202.1 dB. However, a log-fitted curve provides a better fit to the data (i.e. the highest regression coefficient) for calculating *Eigil's* auditory recovery function. By applying this function the animal's hearing sensitivity would have recovered back to the level of the TTS criterion in 55.0 hours after the exposure.

3. BEHAVIORAL REACTIONS

Eigil showed no behavioral reaction during the first exposures when he was exposed to a received pressure levels of up to 174 dB_{pk-pk} re 1 μPa or an SEL of 145 dB re 1μPa²·s. At higher received levels, the animal showed a typical aversive reaction at the time of the sound exposure and behavioral avoidance in the direction of the location of the source and subsequently avoided approaching the exposure station. It should be noted that the exposure station was deliberately placed at a point of maximum received level within the total

available enclosure. After a temporary threshold shift had been documented and confirmed, the received levels were not raised any higher and no further trials were conducted.

Because one of the female harbor porpoises was pregnant during the exposure period, special measures were taken to protect her and the other animal from unnecessary sound exposures. Both females were kept in a sound-insulated pool and their behavior was continuously monitored during the sound exposures. None of them showed any obvious behavioral reactions during the airgun experiments. The attenuation of the airgun impulses inside their pool was at the order of 30 to 40 dB lower than in the research pool. Moreover, this floating pen was installed at the far end of the research pool, hence more than 25 m away from the monitoring hydrophone at *Eigil's* position during the exposures. Correspondingly, the two females were never exposed to peak-to-peak pressure levels of more than 160 dB re 1 μ Pa.

DISCUSSION

The threshold shifts documented in this study represent the first data of its kind for harbor porpoises. Up to now all assessments of potential effects of anthropogenic sounds on harbor porpoises had to be made based on data from other odontocete species, or even terrestrial animals. Thus, the results of this study provide the first reliable information for the harbor porpoise for air gun (or impulse) exposures. These data, and more from future studies, could serve as a basis not only for defining noise exposure criteria not only for this species but also for deriving group-specific noise exposure criteria for all “high-frequency” cetaceans. The threshold shift levels for the harbor porpoise differ strongly from data on the beluga. In the study by Finneran *et al.* (2002) MTTs was elicited in a beluga at a peak pressure of 226 dB re 1 μ Pa and an acoustic energy of 186 dB re 1 μ Pa²·s for this species. The present study provides more empirical data for high-frequency echolocating species than was available for Southall *et al.* (2007). Thus, the authors suggest that the proposed thresholds should be adapted accordingly.

The analysis of the animal's observed behavioral reactions to the fatiguing stimuli for the first time provides quantitative clues of a behavioral threshold in harbor porpoises. The fact that *Eigil* was swimming away from the location of the sound source only during exposure trials but not during control trials infers avoidance or flight behavior. In a free-ranging animal this reaction might have lasted over a longer period of time than observed in *Eigil*, who calmed down and was back under behavioral control of the trainers after a few seconds when he was sent to subsequent hearing tests. It also remains questionable whether or not the level of 174 dB_{pk-pk} re 1 μ Pa pressure or a SEL of 145 dB re 1 μ Pa²·s can be applied as threshold limit for behavioral reactions to impulsive sounds in harbor porpoises in general as *Eigil* was rewarded for tolerating the intense sound exposures. It seems more likely that this limit varies individually and may be context-specific. An extrapolation of these results to natural conditions has to be made very cautiously as wild harbor porpoises may show a comparable reaction at different levels. So far, the only available data on behavioral reactions in harbor porpoises to impulsive sound have come from observations during the

construction of wind turbines at Horns Rev, Denmark, (Tougaard *et al.*, 2003) where at a distance of up to 15 km a movement directed away from the sound source was observed in the animals. In the BROMMAD study (Gordon *et al.*, 2000), by contrast, no obvious behavioral reactions were observed in free-ranging harbor porpoises in response to airgun exposures. In this context, the results of the present study constitute the first behavioral threshold in harbor porpoises that was measured under controlled acoustic conditions. The resulting data may be used as a first indication of a threshold range for behavioral reactions of harbor porpoises. The disturbing nature of this sound to harbor porpoises at the given intensities is emphasized by the avoidance behavior observed in *Eigil* prior to exposures after the exposure level had passed his behavioral threshold for the first time. The fact that *Eigil* was actively avoiding the monitoring hydrophone showed that he was sensitized. It was a lasting effect as he showed no signs of habituation during the remaining exposures.

The rate of recovery from TTS slowed during recovery periods, suggesting an exponential recovery function. These data suggest that recovery rates are different between harbor porpoises and the previously tested mid-frequency cetaceans. The latter usually recover within minutes or, at a maximum, within two hours from a comparable amount of threshold shift (Finneran *et al.*, 2002; Nachtigall *et al.*, 2003, 2004). This slow recovery of the harbor porpoise's hearing sensitivity also indicates that the third exposure to the airgun stimulus at levels over 200 dB re 1 μ Pa (received level of 201.9 dB re 1 μ Pa) may have been premature as the threshold shift was not yet fully recovered. The documented shift of 15 dB above the TTS criterion therefore could then be considered as a cumulative effect from the two consecutive exposures.

The AEP method is the only available method to conduct comparable studies on wild animals. Those studies are relevant to validate the results from a single captive animal in a larger number of animals at a later stage. The results of this study show, on the one hand, that the AEP method can be successfully applied for auditory studies on harbor porpoises even if the animals are unrestrained like *Eigil*, who was actively swimming and free to leave the experiments at any time. His constant movement during the experiments, on the other hand, caused strong myogenic potentials, which were recorded along with the auditory potentials during the experiments. These myogenic potentials are strong enough to raise the overall neuronal noise level of the recorded potentials. Any masking of the lowest levels of the auditory potentials by other electrophysiological signals, such as the myogenic potentials, could obscure the real lower end of the regression line, hence leading to a 0-crossing of the regression at a higher threshold value.

Probably the most prominent factor that may have influenced the hearing thresholds is the level of background noise in Kerteminde harbor. It is most likely that this broadband noise masked perception of the AEP stimuli by *Eigil*. A similar effect has been found in auditory studies in humans (Parker *et al.*, 1976) and also in harbor porpoises (Lucke *et al.*, 2007). Acoustic events, like boats passing at close distance to the research station, were avoided during the experiments by pausing the session. Nevertheless, it was impossible to conduct the experiments at a consistently low level of background noise. As these conditions varied

within each research session, and with extreme noise events excluded, one may assume that roughly the same overall noise conditions applied for all sessions.

Despite these physical factors affecting the baseline hearing thresholds, the results may also reflect a genuine hearing deficit that *Eigil* either developed due to an unnoticed infection of his auditory system or as a result of previously unmonitored exposure to intense sound or a long-term exposure to sounds, e.g. from the nearby harbor. However, it can be ruled out that the elevated thresholds are the result of ototoxic drugs as *Eigil* is known to have never received such treatments. An age-related hearing deficit is also unlikely as it only occurs at high frequencies. The elevated baseline hearing thresholds here stretch over both high and low frequencies. Further aspects leading to error in estimation of *Eigil's* hearing threshold are the comparatively conservative statistical analysis of the resulting EFR data (F-test) and the use of AEP stimuli which are likely to be shorter than the auditory integration time of the animal's hearing system.

As a consequence of this physiological and physical masking, the measured baseline hearing thresholds cannot be regarded as absolute but should be defined as masked thresholds, and, accordingly, the documented threshold shifts have to be regarded as masked temporary threshold shifts (MTTS). The presence of masking noise may have reduced the amount of TTS measured, as indicated by TTS studies on humans (Humes, 1980) and chinchillas (Ades *et al.*, 1974), simulating a pre-exposure reduction in hearing sensitivity. Nevertheless, the onset level of TTS itself, as defined in this study, is likely to be unaffected by the masking noise (Finneran *et al.*, 2005; Southall *et al.*, 2007), presumably due to its comparatively low acoustic energy in comparison to the intense airgun stimuli.

Whether the differences in TTS levels between harbor porpoises and the marine mammal species tested so far are species-specific or representative of the functional hearing groups, as defined by Southall *et al.* (2007), remains unclear. More harbor porpoises as well as other "high-frequency" toothed whale species need to be tested to elucidate this correlation. As for terrestrial animals (Henderson, 2008), the large difference in acoustic tolerance in toothed whales is likely to be attributable to the physical differences in the conductive apparatus rather than to systematic differences in the inner ear. Anatomical differences in the fine structure of the inner ear (Wartzok and Ketten, 1999; Ketten, 2000) and correlated differences in stiffness of the basilar membrane could account for a lower acoustic tolerance to intense sounds in harbor porpoises compared to the toothed whale species tested so far. Moreover, differences in metabolic processes in the inner ear could potentially mediate the long recovery time in harbor porpoises. In the absence of more detailed information it may be valid to generalize and describe this correlation best by means of a mass dependency in the dose-response function for acoustic effects in toothed whales, as documented by Ketten (2006) for the effects of blast impacts.

The TTS data defined in this study are applicable as baseline for the assessment of all activities that go along with the emission of short, impulsive sounds with regard to harbor porpoises. This includes seismic surveys as well as piling construction, both of which show strong acoustic commonalities despite the complexity of their sound emissions. Underwater explosions, however, should be treated separately in this context due to their specific

acoustic characteristics of the shock wave, which may yield strong auditory effects irrespective of the peak pressure or energy of the impulse.

Seismic surveys, piling operations and several other anthropogenic activities at sea involve the repeated emission of intense impulses at varying repetition rates (e.g. 10-15 s interval for seismic surveys, 2-30 s interval for piling). Marine mammals in the vicinity of these operations will consequently be exposed to multiple impulses. While the TTS values determined in this study apply only to a single exposure to a pulsed signal, the auditory effects will accumulate with repeated exposures to such signals if the interval between subsequent exposures is shorter than the recovery time of the hearing system. So far there is no information available on the underlying summation procedure for marine mammals. For harbor porpoises it seems unlikely that they will stay in the area of such intense sound emissions. Nevertheless, if these operations are started without sufficient time for animals to leave the area where received levels will be above or near the TTS levels (as determined in this study), there is an increased risk of TTS or even PTS. The comparatively slow auditory recovery rate worsens this scenario drastically for harbor porpoises compared to mid-frequency odontocetes.

The results emphasize the need for dedicated studies on the cumulative effects of multiple exposures.

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THE USE OF AN AIR-BUBBLE CURTAIN TO REDUCE THE RECEIVED SOUND LEVELS FOR HARBOR PORPOISES NEAR AN IMPULSIVE SOUND SOURCE

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ABSTRACT

In December 2005 construction work was started to replace a harbor wall in Kerteminde harbor, Denmark. A total of 175 wooden piles of 15 m length and 40 cm diameter were piled into the ground over a period of three months. Up to 430 impacts were needed to drive the individual piles into the ground.

During the same period three harbor porpoises were housed in the Fjord & Bælt, a marine mammal facility, on the opposite side of the harbor (at a distance of 110 m). These animals are permanently housed in an open water net cage for scientific purposes as well as public display. This species is known to have sensitive hearing and all animals showed strong avoidance reactions after the start of the piling activities. This involved rapid surfacing and movement to the far end of the enclosure away from the sound source. As an immediate measure to reduce the sound exposure for the animals a 40 m long air bubble curtain was constructed and operated in a direct path between the piling site and the opening of the animals' semi-natural pool.

As soon as the air bubble curtain was installed and operated, no further avoidance reactions of the animals to the ramming activities were apparent. Nevertheless, systematic observation of the animals' behavior was conducted as long as the pile driving continued to identify possible needs for further action. The sound attenuation effect achieved with this system was determined by quantitative comparison of pile driving impulses simultaneously measured in front of and behind the active air bubble curtain.

INTRODUCTION

The preferred method for installing foundations of offshore wind turbines in German waters is currently impulsive pile driving. This process involves the repeated emissions of intense sound impulses into the water. These impulses reach sound pressure levels of 230 dB re 1 μ Pa in 1 m (Parvin *et al.*, 2006), thus having the potential to cause negative effects in the marine environment (Madsen *et al.*, 2006). During the installation of the wind turbine foundations the marine fauna at a wind farm site will be exposed to these sound emissions repeatedly and over an extended period of time (Tougaard *et al.*, 2003). Currently the installation of numerous wind farms consisting of several thousand wind turbines in total is planned in the North Sea. For the German waters alone, permits for over 1,500 wind turbines have been granted already (BSH, 2008).

These waters are inhabited by numerous marine organisms capable of perceiving acoustic stimuli, ranging from invertebrates to marine mammals (e.g. Packard *et al.*, 1990; Fay, 1988; Popper *et al.*, 2001, 2003). Marine mammals, as well as several fish species have developed an acute sense of hearing, but primarily toothed whales depend on this sensory modality for their survival. The only toothed whale species inhabiting German waters is the harbor porpoise. Their functional hearing range reaches at least from 0.5 kHz to 160 kHz (Andersen, 1970; Kastelein *et al.*, 2002). No information on the acoustic tolerance of harbor porpoises is available yet, but on the basis of data on other toothed whale species (Finneran *et al.*, 2002, 2005; Nachtigall *et al.*, 2003, 2004) concern was raised that the exposure to intense pile driving impulses may impair their hearing capabilities.

Therefore, along with ongoing research on the acoustic tolerance of harbor porpoises to impulsive sound, mitigation measures are discussed to reduce the emitted sound energy during the installation of the wind turbine foundations. Besides providing an effective reduction of emissions, viable methods would also need to be practical and cost effective. Air bubble curtains could present a realistic solution.

Two primary mechanisms play a role in the attenuation of sound in bubbly water. Air and water have strong impedance mismatch, with the water-to-air boundary acting as a pressure relief surface and therefore near-perfect reflector. Thus, a cloud of bubbles acts as a series of high-impedance scatterers. For an incident sound field the scattering results in a net reduction of sound intensity along the original transmission axis. Secondly, the incident energy can cause bubbles to resonate, effectively absorbing energy directly. This effect depends on wavelength of the incident energy and bubble size. The presence of the bubbles in the water effectively lowers the sound velocity in this region through reflection, refraction and absorption. The absorption effect is strongly dependent on the ratio between wavelength of the sound and bubble size. As soon as the wavelength exceeds the bubble diameter, the attenuation effect diminishes. However, attenuation efficiency can be improved by increasing the total amount of air per unit time released into the water and hence scattering effects. Air bubbles also expand while rising to the surface due to the decreasing water pressure. They also tend to fuse to larger bubbles. Under normal conditions, an air bubble curtain would

contain bubbles ranging from slowly rising microbubbles to large bubbles of several centimeters in diameter.

Air bubble curtains have already been tested in several projects (Würsig *et al.*, 2000; California Department of Transportation, 2001; Reyff, 2003a, 2003b; Vagle, 2003; Rodkin and Reyff, 2007). However, so far those systems were either extremely expensive and logistically challenging or did not provide sufficient sound reduction. The aim of this study is to develop and test an inexpensive, practical and efficient sound reduction system.

METHODS

The construction work to replace a harbor wall was started in Kerteminde harbor in December 2005. A total of 175 wooden piles of 15 m length and 40 cm diameter were piled into the ground using a 1,400 kg hydraulic pile driver. Up to 430 impacts with an interval of 1.2 s on average between successive impacts were needed to drive the individual piles into the ground. The piling activity was conducted in the south-western part of the harbor over a period of 3 months on an irregular schedule of typically four work days per week and occasional breaks of up to two weeks.

During this time, three harbor porpoises were permanently housed in the “Fjord & Bælt” (F&B) on the northern side of the harbor (see figure 1). Their enclosure is a semi-natural outdoor-pool along the fishing harbor of Kerteminde. Its dimensions are 20 m by 30 m, with an average depth of 4 m. While the long sides are formed by concrete and steel walls, both shorter ends of the enclosure are separated from the harbor by nets thus providing a permanent water exchange and an unobstructed sound coupling with the adjacent harbor.

With the piling activity slowly moving upstream, the distance from the pile driver to the harbor porpoises enclosure increased from 110 m to 175 m. The harbor porpoises showed strong avoidance reactions after the start of the piling activities. This involved rapid surfacing and movement to the far end of enclosure, away from the sound source and an abnormal resting behavior at the surface at the far end of their enclosure over several minutes. As an immediate measure to reduce the sound exposure for the animals an air bubble curtain (ABC) was constructed and operated in a direct path between the piling site and the adjacent opening of the animals’ semi-natural pool.

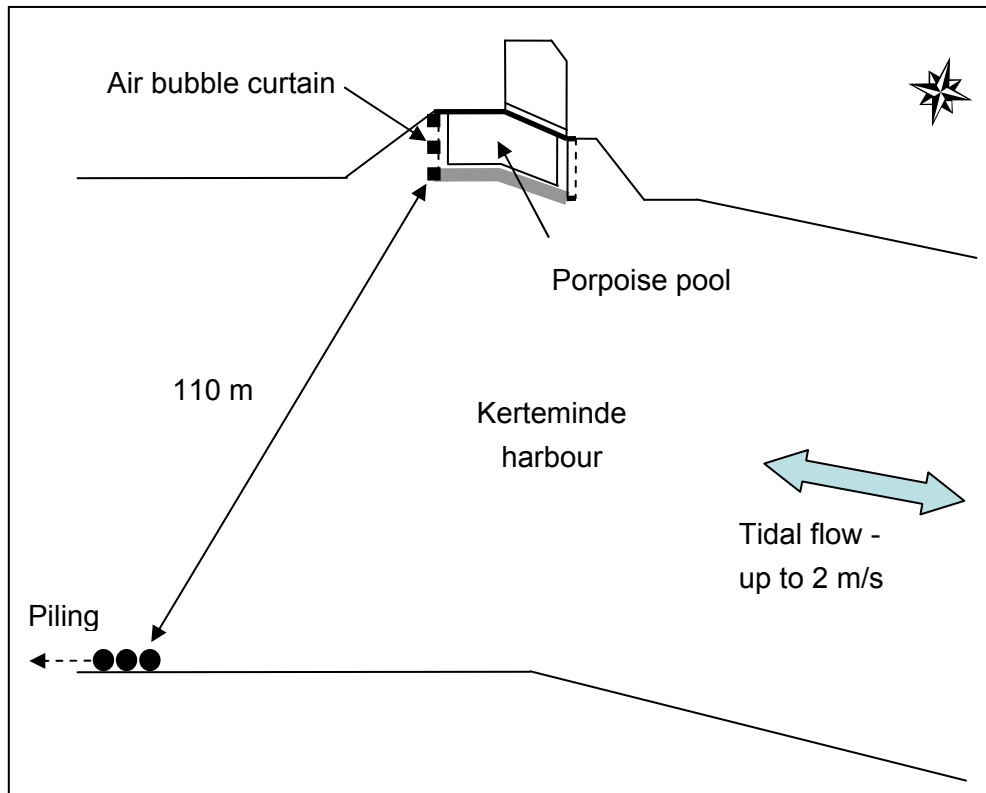


Figure 1. Schematic overhead view of Kerteminde harbor with the Fjord & Baelt located on its northern side and the piling activity (indicated by black dots and arrow) carried out on the southern side. Due to tidal influence from the Great Belt currents of up to 2 m/s can occur in Kerteminde harbor. The dotted line indicates the position of the air bubble curtain. The solid black and gray lines indicate the closed sides of the enclosures, the dashed lines show the position of the nets.

The piling activity was suspended for the duration of the construction and installation of the ABC system. The system had a total length of approximately 50 m and was constructed using 40 mm outer diameter semi-rigid plastic hose (2 mm wall thickness). 2 mm diameter holes were spaced 100 mm apart along its length. The hose was laid on the seabed perpendicular to the sound path (figure 2), along the eastern side of the porpoise enclosure (i.e., the side facing the piling site) for 20 m at a depth of 3-5 m depending on the tide (± 1 m). A maximum tidal current of up to 0.5 m/s was measured in the water column at the eastern side of the animals' enclosure. Due to this current, a double-hose design was chosen and the second half of the hose was doubled back at approximately mid-water (1.5-2.5 m depth) along the same path. This ensured that air bubbles formed a uniform surface in relation to the incoming sound wave and decreased the effect of dispersion due to the water currents as the bubbles rise. The doubling of the hose also increased the total amount of air available within the 25 m aperture across the enclosure to form the ABC. A large compressor was used to provide air at a flow rate of 5 cubic meters per minute at a 5 bar pressure. The compressor was placed approximately 40 m away in a nearby parking lot and was not expected to contribute directly to noise in the water column.

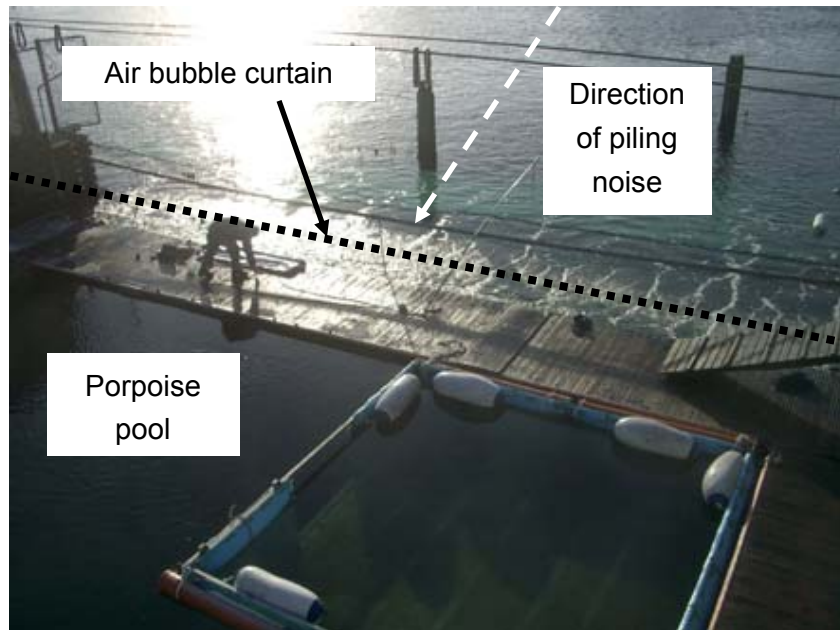


Figure 2. Air bubble curtain deployment in relation to enclosure and holding pool. The air bubble curtain's position is indicated by the black dotted line and the direct sound direction from the piling site by the white dashed line.

An air pressure gradient was found inside the hoses, with the highest pressure at the lower hose next to the landside (closest to the compressor), decreasing towards the harbor wall and lowest at the upper hose at land-side. Due to the bending back of the hose, this bi-directional gradient led to a relatively even distribution of air bubbles along the bubble curtain.

Moderately strong currents (0.5 ms^{-1}) were observed at the position of the ABC system both during flood and ebb tides. These currents resulted in a larger dispersion of the bubble field from the lower line and more drifting as the current had more time to act on the rising bubbles. The displacement and dispersion of the lower bubble field swung left and right of the upper field depending on the state of the tide. A current meter was used immediately before and after acoustic trials to assess the potential curtain drift.

To inspect the bubble curtain and assess the air bubble field at various points during acoustic tests, an underwater camera was deployed within the bubble field. Figure 3 shows a view along the upper line. The ruler was placed 30 cm in front of the camera lens. The wide distribution of bubble sizes and shapes is evident, particularly close to the outlet jets.



Figure 3. Upper bubble field viewed along hose line. Scale 30 cm from lens.

An assessment of the bubble field's state is clearly critical to any acoustic properties measured. In practice, however, the field is highly variable in bubble size, shape, coverage, etc. and depends on input air flow and air pressure, outlet depth, outlet mechanism (lots of small holes or wider spaced bigger holes), currents and bubble rise depth.

PILING AND AIR BUBBLE CURTAIN NOISE ASSESSMENT

Two identical hydrophones were deployed 1 m from either side of the air bubble curtain to determine its sound attenuation efficiency. Both hydrophones were placed in a direct line to the source approximately perpendicular to the air bubble curtain at 2 m water depth (i.e. approximately mid-water depth). Simultaneous recordings of piling impulses in front of and behind the bubble curtain were made for all three sequences. The range (110 m) from bubble curtain to piling source was assessed using a laser range finder.

Received level estimates of the piling noise were made just outside the net cage in the direction of the piling source at a point of the enclosure closest to piling. Measurements were made using calibrated Reson 4033 hydrophones with additional 30 dB of gain (Etec B1501). The signals were recorded directly to hard disc using a National Instruments DAQ card 6062E at 12 bit resolution and a 320 kHz sample rate and a custom software package (Pavan *et al.*, 2001). The preamplifiers were band passed from 10 Hz to 180 kHz. All data were initially checked using commercial sound editing software, then selected sequences were analyzed in detail using custom MATLAB scripts.

BEHAVIORAL OBSERVATION

Concurrently to the installation and operation of the ABC, the harbor porpoises' behavior was observed during and after the pile driving period. The method used was a focal sampling (Altmann, 1974) of all three animals for 10 min. A set of behaviors chosen as indicators of stress, the breathing rate and the surfacing position were recorded for each animal during the sampling period.

The behaviors recorded were:

- Breathing: Every time the animal comes to the surface to take a breath
- Logging: The animal stays motionless parallel to the water surface.
- Porpoising: The animal swims rapidly, clearing the whole body out of the water

The animals' enclosure was divided into three imaginary compartments (A, B and C) of equal size, with A being closest to the piling site (figure 6). The observations were conducted visually, several times a day, by an observer placed on a high point on the side of compartment B.

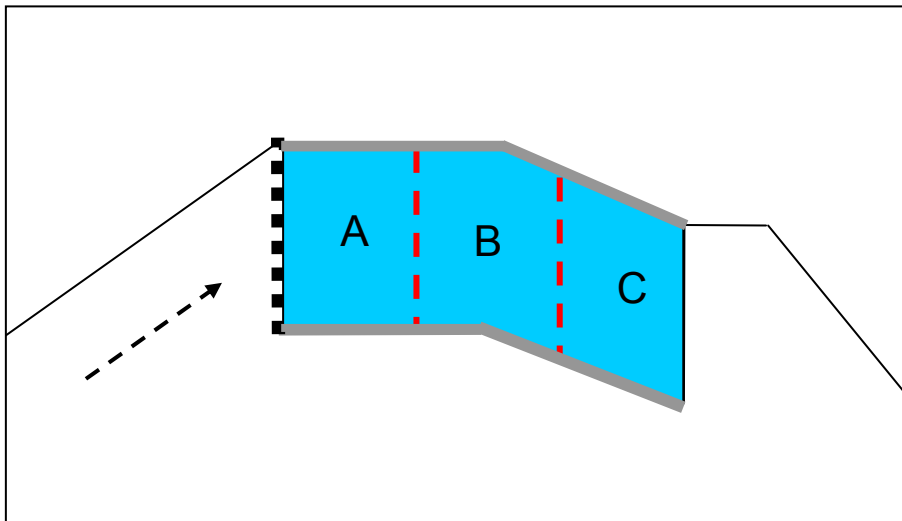


Figure 4. Schematic overhead view onto the porpoise pool with imaginary separation into three compartments (A, B and C). The position of the air bubble curtain is indicated by the black dotted line, the direct sound path of the pile driving impulses by the dashed arrow.

Four conditions were identified for the independent variable (given with their abbreviation):

- Air bubble curtain not operational – no pile driving (bc0nr)
- Air bubble curtain operational – no pile driving (bc1nr)
- Air bubble curtain operational – pile driving (bc1r)
- Air bubble curtain not operational – pile driving (bc0r)

Scenario 4 (ABC not active during pile driving – bc0r) could not be observed because the risk of exposing the animals to the pile driving noise again, with its behavioral consequences and potential implications for an impaired hearing, was considered unacceptable. Instead the visual observations made during the first unexpected pile driving sequences when the ABC was not yet in place were taken as a basis for qualitative assessment.

The behavioral data and breathing rates under the different conditions were submitted to statistical analyses to reveal changes during the observation period.

RESULTS

A typical time domain wave form for a piling impact with the air bubble curtain on is shown in figure 6. In this case, a received sound pressure level (SPL) of 176.0 dB re 1 μ Pa (pk) of the combined noise of the piling impulse and the air bubble curtain noise was observed with an equivalent single impulse SEL of 148.4 dB re μ Pa²s based on a 90% energy criteria (SEL₉₀, see Madsen, 2005). The SEL was calculated over a ~50 ms window shown as vertical dashed lines in figure 6.a. These lines represent the 0-100% analysis band. Over three piling sequences (piles #50, 51 and 52) a relatively high level of variation in received levels was determined. The maximum level was observed at this location for peak SPL of 183.1 dB re 1 μ Pa (pk) with an equivalent SEL₉₀ of 154.0 dB re μ Pa²s. Across three piling sequences (piles nos. 50, 51 and 52) mean levels of 178.2 dB re 1 μ Pa (pk) with a standard deviation of 3.4 dB and 150.5 dB re μ Pa²s with a standard deviation of 3.7 dB were observed for peak received SPL and SEL₉₀ respectively. The air bubble curtain's self-noise and the animals' behavioral response to it were also estimated.

Due to the sound attenuation from the air bubble curtain and its self-noise only a limited number of piling impulses (9) could be clearly identified in the three sequences simultaneously recorded in front of and behind the air bubble curtain. The comparative spectral analysis of a received piling impulse in combination with the self-noise of the air bubble curtain, the self noise alone and the background noise is shown in figure 5. Their equivalent power spectral density is displayed which was integrated over the same (~50 ms) period as the SPL/SEL calculation. A 2048 point Fast Fourier Transformation (FFT) with a 75% overlap was used providing a frequency resolution of ~156 Hz.

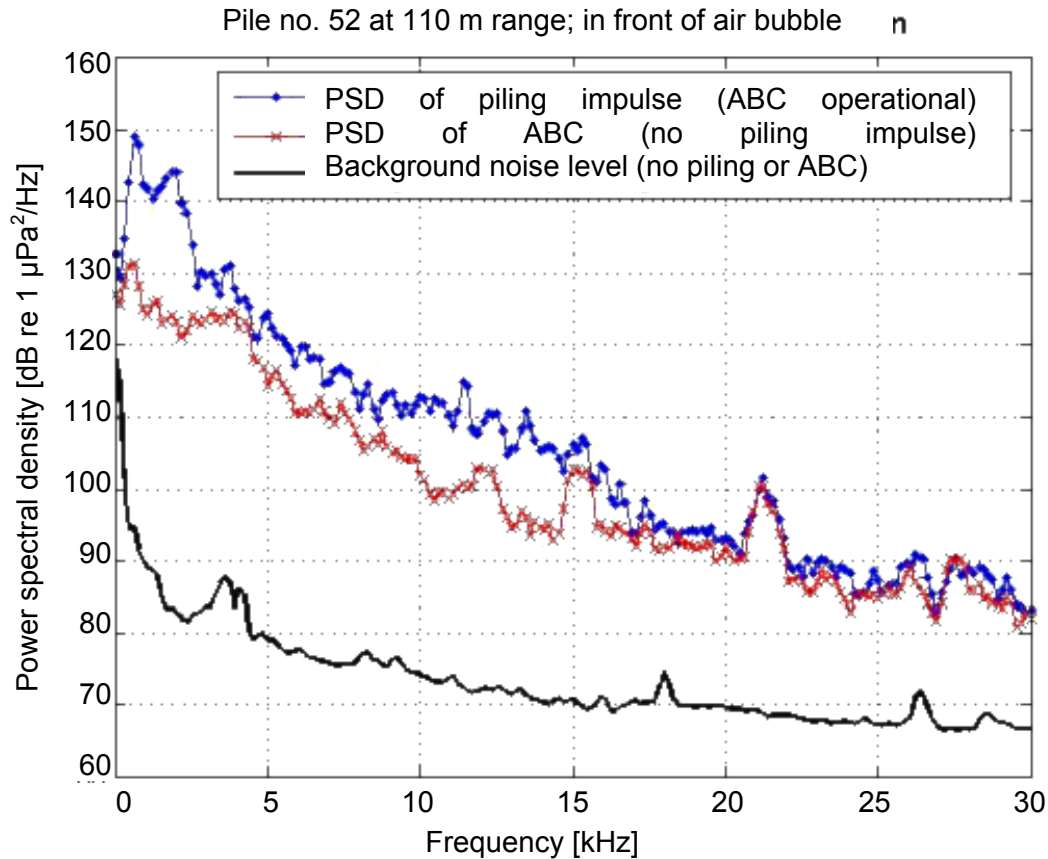


Figure 5. Equivalent power spectral density for pile signal shown in figure 6, period immediately prior to signal and period without pile or air bubble curtain. All three signals were integrated over a 50 ms period, the width of the analysis window was 156.25 Hz.

The upper trace (blue dots) shows the equivalent power spectral density of a single pile driving impulse and of the air bubble curtain's self noise. The peak energy of the impulse was observed at frequencies around 625 Hz. This level is approximately 18 dB above the equivalent level on the second red trace (crosses) calculated from the waveform ~50 ms immediately before the impact. Between 500 Hz and approximately 2500 Hz an average increase (across the integration period) of approximately 18 dB was observed during the impact compared to just before the impact. Additional increases in energy during the impact are also observed as varying levels for frequencies up to approximately 25 kHz. Above this, little differences between the piling noise level and pre-piling noise level were observed while the air bubble curtain was activated. It should be noted that with a repetition rate of the pile driving of 1.5 s in a shallow harbor (2-6 m deep) some residual energy from previous piles may exist in the pre-piling noise analysis window but this is likely to be at least 33 dB lower (assuming cylindrical spreading for a 2.25 km path length) than the initial impact. The third trace (black solid line) shows the background level recorded just before the air bubble curtain was activated and before piling commenced. The air bubble curtain caused a marked increase in the general noise level, ranging from ~12 dB at 30 kHz to around ~40 dB at 2.5 kHz. At frequencies above approximately 15 kHz the noise generated by the air bubble curtain reached spectrum levels comparable to the pile driving impacts. The distance from

the hydrophone to the outlet jets of the midwater line was approximately 1 m. A peak between 20 and 22 kHz with a averaged peak level of around 100 dB re $\mu\text{Pa}^2/\text{Hz}$ at 21 kHz was observed in both air bubble curtain scenarios but not in the background scenario. In all three case identical integration periods and analysis were used.

ATTENUATING EFFECT OF AIR BUBBLE CURTAIN ON PILING NOISE

Individual pile impacts were then compared both inside and outside the air bubble curtain. Figure 6 shows an example of received sound pressure levels from simultaneous recordings made inside and outside the air bubble curtain from pile 51. In this case, the peak received level is 181.8 dB re $1\mu\text{Pa}$ (pk) and SEL_{90} is 153.4 dB re $\mu\text{Pa}^2\cdot\text{s}$. Inside the curtain the peak level has dropped to 165.2 dB re $1\mu\text{Pa}$ (pk) and the SEL_{90} to 136.7 dB re $\mu\text{Pa}^2\cdot\text{s}$. This is a reduction in received level across the air bubble curtain of 16.6 dB and 17.0 dB for peak and SEL_{90} levels, respectively.

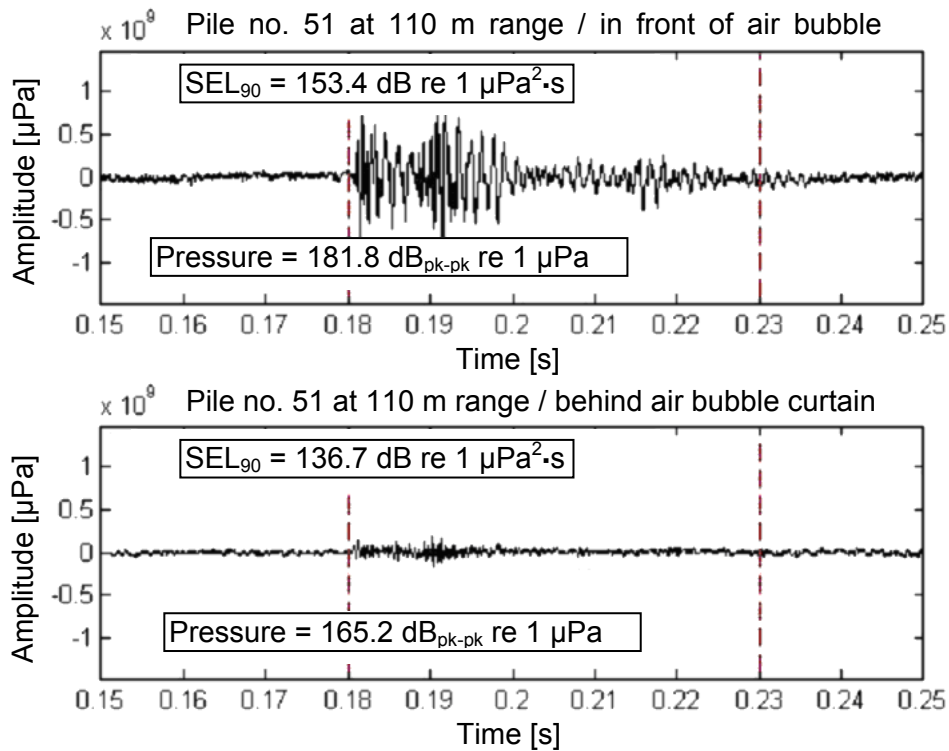


Figure 6. Comparison of waveforms of a typical piling signal recorded simultaneously inside and outside an air bubble curtain at a distance of 110 m from the piling site. The received sound pressure levels and sound energy are given for both recordings. The vertical dashed lines indicate the limits of the ~50 ms window over which the 90% criterion for the sound energy was integrated. The hydrophones were positioned at a 1 m distance from the air bubble curtain at a 2 m water depth.

Additional spreading losses recorded at the hydrophone inside the air bubble curtain were considered negligible compared to the hydrophone outside the curtain and closer to the pile. This was estimated using the difference in cylindrical spreading from 109 m to that at 111 m giving a difference of less than 0.1 dB. The results of all nine piling impulses identified in the simultaneous recordings in front of the bubble curtain and behind it are listed in table 1.

Table 1. Peak pressure and sound exposure levels and differences in front of and behind (relative to the sound source) the air bubble curtain (ABC) for nine impacts across three piling sequences (pile nos. 50, 51 and 52)

	In front of ABC	Behind ABC	Difference	In front of ABC	Behind ABC	Difference
pile no.	Peak to peak sound pressure level [dB _{pk-pk} re 1μPa]			Sound exposure level [dB re 1 μPa ² s]		
pile 50 4th	178.7	161.0	-17.7	152.9	133.7	-19.2
pile 50 9th	180.6	163.3	-17.3	153.7	134.9	-18.8
pile 51 2nd	176.6	166.0	-10.6	149.1	134.4	-14.7
pile 51 6th	181.8	165.2	-16.6	153.4	136.4	-17.0
pile 51 14th	177.2	165.7	-11.5	151.3	137.2	-14.1
pile 51 17th	183.1	168.6	-14.5	154.0	139.0	-15.0
pile 52 2nd	171.8	154.7	-17.1	142.5	129.6	-12.9
pile 52 23rd	176.0	161.0	-15.0	148.7	133.7	-15.0
pile 52 27th	178.4	159.0	-19.4	148.6	132.8	-15.8

Figures 7 and 8 shows the peak pressure and equivalent sound exposure values for nine impacts taken across the tree pilings sequences studied.

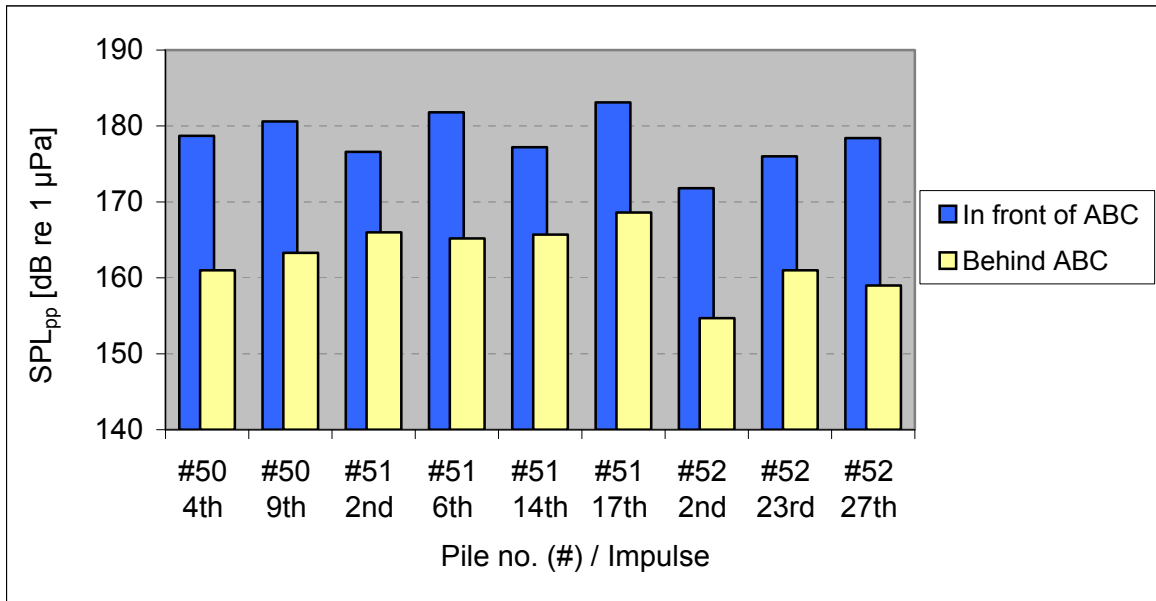


Figure 7. Peak-to-peak pressures measured in front of and behind (relative to the sound source) the air bubble curtain (ABC) for nine impacts across three piling sequences (pile nos. 50, 51 and 52).

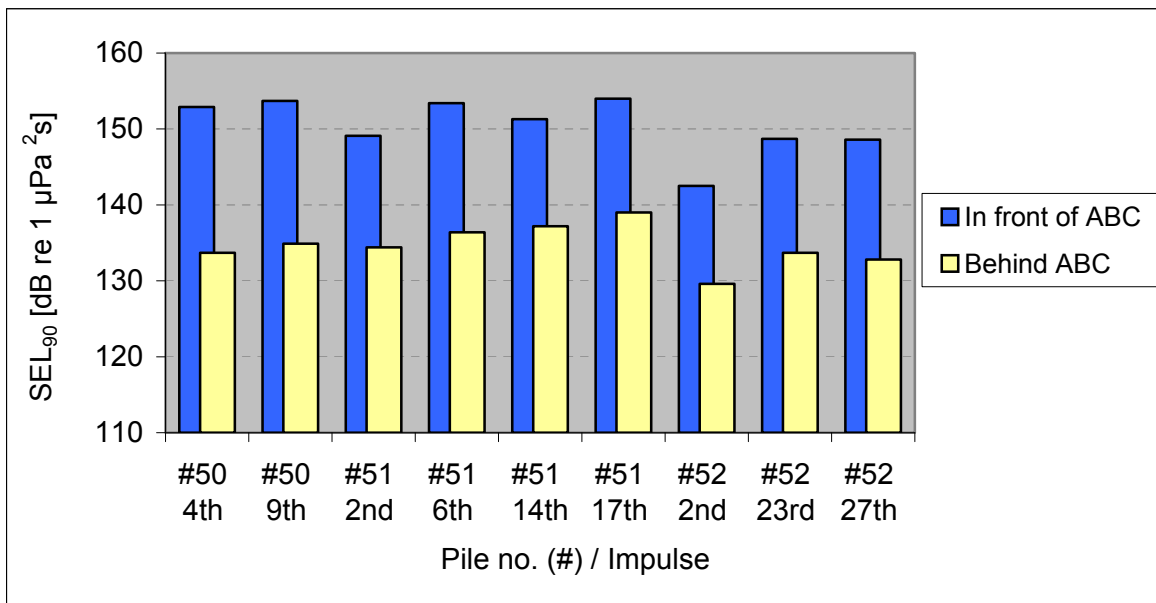


Figure 8. Sound exposure levels measured in front of and behind (relative to the sound source) the air bubble curtain (ABC) for nine impacts across three piling sequences (pile nos. 50, 51 and 52).

The attenuation of the ramming impulse noise was observed in the range of 11 dB to 19 dB in signal peak-to-peak and 13 dB to 19 dB in the SEL with the use of the air bubble curtain over multiple piling sequences. In two thirds of the cases a stronger reduction was seen in the calculated SEL than the equivalent peak level. However, in three cases the peak pressure was more attenuated than the SEL. This may be due to the difficulties of peak-to-

peak level measurement in a shallow water environment where constructive and destructive interference of multi-path signals can result in rapid changes in received level.

BEHAVIORAL REACTION

With the onset of the piling activity, the three harbor porpoises in the enclosure immediately displayed strong behavioral reactions such as speed swimming and porpoising for as long as the piling continued. These behaviors were interpreted as avoidance reactions to the sound exposure as they had been observed in these animals during exposures to novel acoustic stimuli before and are similar to flight behavior observed in free ranging animals. These energetically demanding behaviors were in some cases followed or interrupted by periods when an animal could be observed logging at the surface at the far end of their enclosure. The breathing frequency was elevated during the piling sequences when no ABC system was in place and operational.

Due to the unexpected onset of the piling activity no quantitative observation of the animals' behavior was conducted prior to this study. However, visual observation of the animals' overall behavior, and their breathing rates in particular, had been conducted routinely, thus providing a useful indication of normal behavior.

Systematic observation of the porpoises revealed no further obvious avoidance behavior in response to the ramming activities. The only avoidance reaction observed occurred when the bubble curtain was initially activated. This reaction, however, faded within the first few days whenever the air bubble curtain was active.

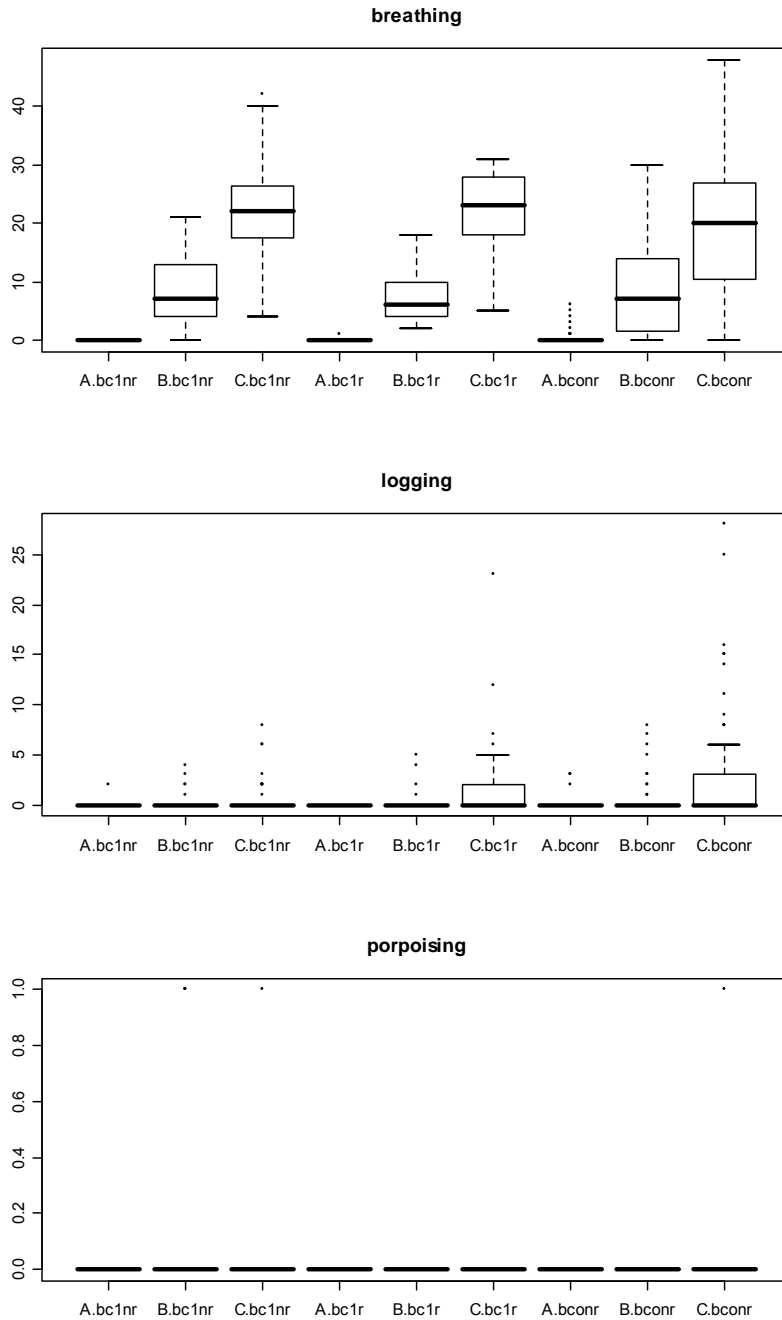


Figure 9. Boxplots of results from behavioral observations of the three harbor porpoises at the Fjord & Bælt. The frequency of occurrence of the selected behaviors (as dependent variables: breathing, logging and porpoising) in the three imaginary compartments of the porpoise enclosure is analyzed for each scenario separately (bc0: air bubble curtain not operational, bc1: air bubble curtain operational, nr: no pile driving, r: pile driving).

The results clearly show a preference by the three animals for compartment C, which is the furthest from the pile driving site. This effect is most obvious with regard to breathing, which also indicates the animals' spatial preference within their pool. Only slight differences can be found in the occurrence of the behaviors between the three scenarios, but none are

significant. Most obvious is the complete absence of porpoising in all compartments and scenarios. Logging behavior – also considered a reaction to the pile driving – occurred only a few times, again only in compartment C at the far end of the porpoise pool (relative to the pile driving). There is no indication of a potential behavioral effect induced by the operation of the air bubble curtain. These results differ qualitatively from the observation during the fourth scenario (no bubble curtain during pile driving activity) when all three animals showed repeated porpoising and increased breathing in all three compartments over an extended period of time.

DISCUSSION

The results of the acoustic and behavioral data analyzed in this study show that the air bubble curtain can be an effective measure to mitigate sound-induced effects on marine mammals. The work presented here resulted as immediate reaction to the commencement of piling activities in Kerteminde harbor, and the bubble curtain was constructed with the best available knowledge of its potential attenuation effects. The results presented show that attenuation of this type of piling noise was achieved in this case, both in terms of the peak observed signal and the sound exposure level. In the former case attenuation ranged from 11 to 19 dB in signal peak-to-peak and 13 to 19 dB in the impulse energy (SEL). A marked increase in the general noise floor due to the bubble curtain noise was also observed, with levels nearly 40 dB higher at some frequencies. However, these levels were still below the attenuated signal 100 m from the piling source suggesting that the bubble curtain was indeed helpful in reducing the effects of piling noise on the captive porpoises.

Furthermore, the porpoises generally resumed normal activity during piling when the bubble curtain was activated. Porpoising and speed swimming, along with an increased breathing rate, are likely indicators of increased stress in the animals. No behavior of this type was observed after the bubble curtain was installed. Because the behavioral response of the porpoises without the bubble curtain present could not be tested due to safety consideration, no definitive conclusion of mitigation benefit could be drawn from behavioral data. For example, additional factors such as habituation to the piling noise over time and reduced sound levels as the piling moved away from enclosure could also influence observations. It seems likely that the measured attenuation effects of the ABC play a significant role, although the opportunity to independently test the factors was not available during the study period.

The fact that the animals were logging at the surface at the far end of their pool (compartment C) may be due to a potential zone of reduced sound pressure. Such effects can be caused by acoustic near-surface effects (Urick, 1983) as well as constructive and destructive interferences within the uneven geometry of the porpoise pool. These effects are likely to occur in shallow waters with different reflecting or obstructing structures and may have led to a decrease in received sound pressure levels in this area by several dB.

With regard to the animals' apparent general preference for the far end of their pool, it must also be considered that the F&B is a public facility. There are several shows daily displaying the harbor porpoises. The shows involve repeated feeding and occur mainly in compartment C, rather than A or B. The positive reinforcement at this end of the pool, along with fact that the trainers' office and the so-called fish kitchen are also situated at the far end of compartment C, may have biased the surfacing position of the animals. As no quantitative observations were made in this respect prior to the pile driving, this cannot be assessed quantitatively.

A study on the tolerance of the animals hearing to intense impulsive sounds revealed that harbor porpoises are likely to show a temporary threshold shift (TTS) in their hearing sensitivity at a received sound pressure level above 200 dB_{pk-pk} re 1 μPa or a received sound energy above 164 dB re 1μPa²s (Lucke *et al.*, 2008). A reduction in sound level by 16 dB on average – as achieved with this system – would reduce the range of this effect around a sound source by over 75% and the affected area by over 90% (assuming spherical spreading with a propagation loss of 20·log(r), with r = range in meter).

The values determined by Lucke *et al.* (2008) refer to single exposures to impulsive sounds. When the pile driving activity in the harbor of Kerteminde initially began without any mitigation measure in place, the animals were exposed to several hundred pile driving impulses at comparatively high sound pressure levels. Even though the level of individual impulses may have never reached the TTS threshold the animal may still have suffered from TTS due to the summation of the exposures, i.e. due to a cumulative effect within their auditory system.

Of course, the effectiveness of such an air bubble system in open waters based on the results of this study remains difficult to assess. With stronger currents and greater water depths in which such a system could possibly be installed the efficiency of the present design may have to be optimized. Such changes, however, may neither add much cost to the system nor make it less practical. One beneficial aspect of the chosen design was the installation of the bubble curtain near the sound receivers (the animals), thus avoiding the problem of bottom propagation to some extent. This transmission of some sound energy through the bottom (like with pile driving) may contribute substantially to the received level at the animals due to reemergence of the sound back into the water column behind the bubble curtain. This effect of sediment borne energy on received level in the water column would potentially be worse as the sound barrier is placed nearer to the source, giving more time for the sediment borne energy to reemerge into the water column.

It also remains unclear to which extent the behavioral reaction of harbor porpoises held under human care in a comparatively noisy fishing harbor can be used as proxies for wild animals. Nevertheless, any attenuating effect would result in a smaller range around a sound source in which behavioral reactions would occur. Tougaard *et al.* (2003) observed a significant increase in directional movement of wild harbor porpoises away from a site (Horns Rev, Denmark) where foundations were driven into the ground by using impulsive pile driving. This supports the interpretation of the speed swimming and porpoising as avoidance behavior in the captive harbor porpoises. Such reactions imply that the animals changed

their behavior from whatever they were occupied with before and that they are leaving their habitat. Depending on the importance of the behavior and habitat this can have serious consequences, ranging from reduced time to conduct important behaviors (such as feeding, breeding and nursing) to the permanent loss of an important habitat. Tougaard *et al.* (2003) detected the presence of harbor porpoises in the same area within a few hours of the pile driving ending. However, as the site is within an area with high densities of harbor porpoises (Hammond *et al.*, 2002; Tougaard *et al.*, 2006), it remains uncertain whether or not the same animals returned (which leads to the assumption that it may have been an important habitat) or that animals from an adjacent area that had not been exposed to the pile driving sounds at high intensities moved into the area. This latter explanation is in agreement with results from a site in the Baltic (Nysted, Denmark) where harbor porpoise densities are lower (Hammond *et al.*, 2002; Scheidat *et al.*, in press). There, the pile driving activity at a single foundation was followed by a decreased density and habitat use by harbor porpoises (Henriksen *et al.*, 2003), an effect that persisted for at least two years (Tougaard *et al.*, 2005). In contrast, the piling activity at the first site (Horns Rev) occurred repeatedly and comprised the installation of 80 foundations over a period of several months. Gordon *et al.* (2000) conducted a controlled acoustic exposure experiment in an area with a comparable high harbor porpoise density. Their study did not reveal any changes in distribution of the harbor porpoises in response to exposure to seismic airgun stimuli. The data collected in their study were to measures of presence or absence of harbor porpoises in the ensonified area. Conclusions on behavioral reaction can thus not easily be drawn from these data. However, they conclude that the bay may have been a preferred habitat for the animals and that lack of any significant changes in distribution of harbor porpoises in the presence of airgun sounds may be attributed to this.

With the planned installation of tens of wind farms in the German waters of the North Sea alone with a minimum several hundred wind turbines over the next years this would increase the percentage of the whole population that could be affected at least once. Moreover, it is likely that individual animals would be exposed repeatedly to pile driving sounds. This could occur if the animals return to the same site and experience the sound emissions from the installation of the next foundation or move to other areas where a different wind farm is build.

So far there is no data available for any marine mammal species on cumulative effects from repeated exposure to intense sounds. However, the TTS study in harbor porpoises (Lucke *et al.*, 2008) provided first data on the recovery time of the animals' auditory system after exposure to impulses above its TTS threshold. This indicates that harbor porpoises need long periods (up to days) to recover from TTS. Any repeated exposure within this period would lead to a summation effect and aggravate the auditory effect. Furthermore, cumulative non-acoustic effects could potentially also arise from acoustic effects in conjunction with other environmental stressors such as pollution and food depletion.

The results presented here show that air bubble curtains can provide the opportunity to reduce acoustically induced detrimental effects at least to some degree. Any attenuation effects would help mitigate overall received levels around a noise source reducing overall impact on the marine environment. Any effective reduction of source level also has the

potential to reduce overall impact zones (ranges from source for received levels that may cause various responses) more efficiently than simple range exclusion and use of natural attenuation of sound energy with range. The latter is because a reduction at source is uniform with range however the effect of spreading losses are significant at shorter ranges and becomes less so a greater distances.

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SYNTHESIS

The baseline auditory studies on harbor porpoises and harbor seals (chapter 1 and 2) both show that the AEP method is now well established and can be used under controlled conditions, as well as in the field, to achieve improved auditory data sets on both species. The accordance of the auditory data measured in the present studies compared to the published data indicates that thresholds measured so far are representative for these two species. However, two factors were shown to potentially influence the quality of the results and were still not sufficiently controlled to achieve absolute hearing thresholds:

1. The influence of background noise on the perception of the acoustic test stimuli; Especially in tests conducted in the field, it is almost impossible to reduce the noise to levels required to achieve absolute hearing thresholds, as could be achieved under laboratory conditions.

However, the underlying objective of the studies was to assess the effects of anthropogenic noise on animals in their natural environment. Any sound-induced effect would occur in the presence of an ambient noise level “normal” for the particular area. To account for effects of varying levels of ambient noise between different auditory tests, future studies will need to determine the auditory parameters (like the critical ratio) relevant for assessing acoustic masking effects. It is essential to document the ambient noise level during auditory testing.

2. The animals’ movements during the test procedure. Dissociatives were successfully used in harbor seals to reduce movements, a comparable method has yet to be found and tested in harbor porpoises. Immobilization is a key prerequisite for auditory testing in wild seals. For harbor porpoises, it may be possible to administer a mild sedative as long as the animal is under good medical control during the whole procedure. This would most likely reduce the amount of myogenic potentials and lead to comparable thresholds in all harbor porpoises tested, even in the field.

It should be noted that auditory measurements were also conducted on a wild gray seal for the first time. During a seal catch intended to focus on harbor seals a gray seal pup was caught. This opportunity was used to test if the AEP method is applicable in this species similarly to harbor seals.

The initial measurements comprised tests to find the best placement for the electrodes. As in harbor seals the best signal-to-noise ratio for the AEPs was achieved with the cephalic electrode at a position 2 cm forward of the ears on the dorsal midline. The animals’ hearing sensitivity was measured at three frequencies (2 kHz, 4 kHz and 8 kHz) before the animal had to be released due to medical indications. The resulting thresholds were all elevated compared to the highest threshold measured in a harbor seal in the study presented within this dissertation. At 2 kHz a hearing threshold of 64.3 dB_{pk-pk} re 20 µPa (+5.6 dB) was measured, at 4 kHz the threshold was 36.6 dB (+7.7 dB) and at 8 kHz the threshold was 38.4 dB (+13.7 dB). These data should be regarded as first indicators of the hearing threshold of gray seals. The results show that the method is generally also applicable to this species. The

only auditory data available for gray seals so far were achieved in an invasive electrophysiological study via implanted electrodes (Ridgway and Joyce, 1975). As cortical thresholds were obtained in this study, the results are not comparable with thresholds achieved with AEP measurements. Moreover, the primary focus of the study by Ridgway and Joyce (1975) was not on auditory testing and the setup is not described in sufficient detail to assess potential artefacts resulting from their study design.

The masking study in a harbor porpoise (chapter 3) revealed that the operational noise emitted by offshore wind turbines had only a limited masking effect on the animal's acoustic perception. Prior to this study, concern was raised that a wind farm consisting of tens of wind turbines in a row may have a barrier effect on the harbor porpoises and alter their movement pattern considerably. Based on the results of this study, such an effect is unlikely as masking would only occur over several tens of meters from the turbines and perception of the operational sound would probably be limited to below ~100 m. The measurements were, however, conducted based on the operational sound emitted by a 2 MW wind turbine. Meanwhile, the average size of wind turbines has gone up to 5 MW and may increase even further. The results of an acoustic modelling study indicate that these larger turbines will emit operational noise at higher noise levels into the water column (DEWI, 2004) while the spectral peak of the noise is presumably shifted to the lower frequencies. The difference in emitted sound pressure levels can reach several tens of dB, thus leading to an increase in acoustic masking ranges for harbor porpoises. This effect may be compensated by the shift in peak acoustic energy towards lower frequencies where harbor porpoises have a reduced hearing threshold. A barrier effect remains unlikely as larger wind turbines will be placed at increasing distances from each other. The elevated noise levels will be attenuated over these longer distances between adjacent turbines and likely reach levels below the hearing thresholds of harbor porpoises. However, acoustic recordings of the operational noise of these larger wind turbines as well as dedicated studies on the auditory masking parameters (critical ratio, see above) are required to conclusively evaluate this new situation.

One of the key parameters needed for the regulatory management of anthropogenic activities with acoustic consequences in the German North Sea with regard to marine mammals is temporary threshold shift (TTS). The vital dependence of harbor porpoises on their sense of hearing moved this species to the center of attention. The results of the TTS study on a harbor porpoise (chapter 4) provide the first reliable species-specific data applicable to a variety of sound sources. The sound stimulus used to test the auditory tolerance of harbor porpoises (airgun impulse) is comparable in its acoustic characteristics to pile driving impulses, and results can be extrapolated to any comparable impulsive sounds without substantial reservations. The impact of these two types of anthropogenic sounds is considered potentially significant on an individual as well as a population level, which underscores the need for such information.

Because only one animal could be tested in this study, more harbor porpoises will need to be tested in a comparative TTS study to account for individual variability. Another important factor regarding the effects of anthropogenic acoustic activities are exposures to repeated or long term exposure to sound which can aggravate the TTS effects on the

hearing system. No data on marine mammals are currently available. To quantify these effects it has to be determined if the auditory dose-response function in marine mammals follows an “equal-energy rule,” i.e. how the hearing system would react to multiple or longer exposures to intense signals. The published TTS data on cetaceans are limited to two species, the bottlenose dolphin and beluga (Finneran *et al.*, 2000, 2002, 2005; Schlundt *et al.*, 2000; Nachtigall *et al.*, 2003, 2004) and were achieved for exposures to a single acoustic impulse and to nonpulsed sounds ranging from 1-s to ~50-min duration. As stated by Southall *et al.* (2007) the TTS data from these studies show that the “equal-energy rule” can be applied as the data “*generally support the use of SEL as a first-order approximation, at least until additional data are available*”.

The most likely scenario during the installation of wind turbines or seismic surveys involves an exposure of marine animals in the vicinity to several tens or hundreds of impulses when no precautionary measures are taken. Additionally shipping and other anthropogenic activities produce a continuous noise floor to which the animals may be exposed over extended periods of time. It is shown in terrestrial animals that multiple exposures to sound may lead to cumulative auditory effects (e.g. Hamernik *et al.*, 1987; Ahroon *et al.*, 1996). However, data to assess this effect in marine mammals are not available.

One of the most promising noise mitigation methods with regard to stationary sound sources is the air bubble curtain (chapter 5). It has proven to reduce the piling impulses by an average of 16 dB over a broad frequency band and had significant effects on the behavior of the harbor porpoises. Even though this method and the underlying principle have been known for decades, studies conducted previously did not yield the desired attenuation or were logistically impractical. The system tested in the harbor of Kerteminde, however, has potential to be efficient in offshore waters and could be installed without large logistical efforts. Its efficiency can be improved by adding more lines to the system, i.e. providing a better coverage of the water column with air as sound insulator, by changing the number size of the holes or varying the air pressure in the system.

The results of the studies presented in this dissertation may serve as baseline for a long-term monitoring of the acoustic health status of harbor porpoises and harbor seals. They can also be included in a general assessment of potential effects of specific anthropogenic activities on the two marine mammal species studied. The results are of course not sufficient to answer all questions regarding potential effects of anthropogenic sound on the marine mammals in German waters but rather a starting point.

The information that can be drawn from the studies presented relates to the questions of this dissertation as follows:

- Are seals originating from the North Sea already affected by the sound level in their environment?

This aspect was tested in harbor seals. The auditory data on the wild subadult seals represent hearing thresholds to aerial sound under natural background noise conditions. These levels were comparable to data achieved in psychoacoustic tests on adult seals originating from other populations and sound environments. If the animals tested in the present study would have been exposed to intense underwater sounds prior to the tests, their hearing sensitivity would have been compromised, independent of whether the auditory tests had been conducted in air or underwater. The subadult wild animals had spent at least one year within the acoustic underwater environment of the North Sea before being tested. As their hearing thresholds were in a normal range, the conclusion seemed appropriate that neither the overall underwater (and in-air) noise level nor any individual sound source has reached detrimental levels in terms of the auditory well-being of the subadult seals.

However, these were comparatively young animals and adult seals may already suffer from a chronic hearing impairment because they could have experienced more noise than their hearing system can tolerate. As the AEP method can be regarded established and baseline data exist, the next auditory studies on wild harbor seals should focus on testing adult animals to create a data basis for this age class. Several animals need to be tested to determine a representative audiogram for harbor seals. This data basis would be required to identify hearing impairment in individual seals due to e.g. aging or pathologies in an undisturbed population and due to anthropogenic acoustic emissions in areas like the German North Sea.

The threshold levels measured in a gray seal pup are the first auditory data on a wild individual of this species. Moreover, there are no comparable data on gray seals published at all. Due to this lack of baseline data and with these few data points gathered, it is of course impossible to draw conclusions on the auditory health status of gray seals.

- Is anthropogenic sound likely to interfere with the animals' communication?

This objective was tested in a harbor porpoise held under human care. It had been suggested before that harbor porpoises have a low-frequency component in their echolocation signals which they use for communication purposes. In this study, the focus was on the potential masking effect of operational sound of offshore wind turbines characterized by continuous low-frequency sound at varying source levels. There was growing concern that these emissions could mask the (potential) communication of harbor porpoises.

The results indicate that the type of wind turbines installed so far at sea would only interfere with the animals' perception of signals at short ranges, i.e. at a distance of tens of meters from the turbine. The result can in principle be extrapolated to sound sources with comparable types of sound emissions. However, in the absence of data on further auditory parameters such as the critical ratio (i.e. the level (in dB) at which a pure tone can be

detected above the background or, as in this case, the operational noise level), any extrapolation should be treated with great caution.

Beside the aspect of masked communication, the results gained from the masking study provide valuable information in a broader context. They allow to assess if harbor porpoise perception of other, biological meaningful signals could potentially be masked by the operational noise of the offshore wind turbines. As mentioned, the current design of offshore wind turbines causes masking only at a range of tens of meters. It is unclear if this issue may become more relevant with larger turbines and higher noise levels as the peak in the frequency spectrum will shift towards lower frequencies and turbines will be placed further apart (see discussion above).

- How tolerant is their hearing to exposure to the most common type of intense sound?

This aspect is most important for harbor porpoises as they rely on hearing for their survival. The most relevant sound sources in this context were airguns used during geophysical seismic surveys and offshore impact pile driving. An airgun was used in the study conducted on a harbor porpoise held under human care. The study provided the first data on temporary threshold shift in harbor porpoises. Moreover, behavioral reactions were documented and could also be related to a threshold level. The study was conducted on a single animal (n=1) and the determined threshold values may not reflect the general threshold levels. Nevertheless, the resulting threshold values indicate that harbor porpoises are more sensitive to intense sound exposures than the cetacean species tested so far. For impulsive sounds a difference in sound pressure level of 26 dB and 22 dB in sound energy was determined compared to belugas (Finneran *et al.*, 2002). This may have implications for the noise exposure criteria and the regulations based on them as they. Accordingly, the noise exposure criterion for “high-frequency” cetaceans suggested by Southall *et al.* (2007) would have to be lowered for single pulses by 24 dB in sound pressure level and by 19 dB in sound exposure level. This is not taking into account any weighting function as applied by Southall *et al.* (2007) and would apply only if the levels determined in this study are considered representative. The noise exposure criterion for harbor porpoises proposed by the UBA (2003) in contrast appears appropriate for exposures to single pulses.

- Are there practical acoustic mitigation measures?

This aspect was studied at a facility housing three harbor porpoises which had shown behavioral reactions to the onset of pile driving activities at close range. The only option to reduce the sound exposure for the animals was to install a sound mitigation system. The air bubble curtain proved to be an effective method to reduce the sound immission (i.e. the received sound level) to levels at which the animals returned to their normal behavior.

Stationary systems like the air bubble curtain tested in the present study can be used to reduce the sounds emitted from stationary sound sources. The design of the system can be optimized to increase the efficiency e.g. by adding more lines to system and place them

evenly over the entire water column. The self-noise of the bubble curtain can be reduced by varying the air pressure, the size of the holes or by using a perforated hose. At a more advanced stage these systems may also be installed in offshore areas to reduce the emissions from e.g. offshore pile driving into the surrounding waters.

MITIGATION MEASURES / BEP

The air bubble curtain should not be the only measure taken to reduce the acoustic emissions from piling or other stationary sound sources. In order to mitigate the effects of anthropogenic sound on the marine fauna and especially on marine mammals for every type of activity at sea (stationary and mobile) the best available technique (BAT) should be applied in combination with the best environmental practice (BEP):

Definitions (OSPAR Convention, 1992, Appendix 1):

“Best Available Technique (BAT) means latest stage of development (stage of the art) of processes, facilities or of methods of operation which indicate the practical suitability of a particular measure for limiting discharges, emissions and waste.

“Best Environmental Practice (BEP) means the application of the most appropriate combination of environmental control measures and strategies.”

With regard to the emission of anthropogenic sound and its potential effects on marine mammals, BEP should include the following measures, either individually or in combination, as applicable:

- Zero-Emission (No action): If the consequences for the marine environment are not acceptable at all after balancing all regulations and interests, the activity should be omitted.
- Temporal or spatial closure of critical habitats; in case the activity is planned within a time period or area critical for the marine mammals (or species of concern), the activity should be delayed and/or relocated (Weir and Dolman, 2007).
- Use of alternative methods with lower level of sound emission; this measure refers to the noise related aspect of BAT. Sound-emitting devices or procedures should be improved by either new techniques or devices generating a lower level of sound emissions (Weir and Dolman, 2007).
- Reduction of sound emissions; the source level and/or duty cycles should be reduced to minimum required to meet the objective of the given activity. Redundant sound

emissions should be avoided, thus reducing the overall amount of noise emitted into the water column (JNCC, 2004; NURC, 2006; Weir and Dolman, 2007).

- Use of active mitigation methods; The sound emitted by stationary sound sources can be further reduced by installing an air bubble curtain around it or acoustically disconnecting the sound source from the water through foam or coatings (Schultz-von Glahn *et al.*, 2006).
- Environmental scoping study; prior to operating an intense sound source all available information on the presence of marine mammals should be studied (assessment of existing data on distribution and abundance in the study area). Modelling of such data should be performed if no or insufficient data are available. This should be based on relevant biological and oceanographic parameters to predict the actual encounter risk (NURC, 2006).
- Real-time acoustic and visual monitoring; to avoid an exposure of marine mammals to intense levels of sound, their presence should be monitored in real time through visual observation (from a suitable platform at sufficient height) and a click-detector system equipped with a hydrophone to detect the animal's acoustic echolocation signals. The monitoring should be started at least 30 minutes prior to the start of the sound source (Weir and Dolman, 2007).
- Deterrence; independent of efforts to detect marine mammals in the vicinity of an intense sound source, acoustic deterrent devices (harbor porpoises: "pingers"; seals: "seals scarers") should be deployed to drive animals out of the vicinity of the sound source. These devices have been used both for seals and harbor porpoises and have proven to be effective in scaring the animals away from the source by several hundred meters (Yurk and Trites, 2000; Cox *et al.*, 2001; Culik *et al.*, 2001). Yet, habituation is possible (Cox *et al.*, 2001). Furthermore, since these devices emit sounds at high source levels (Lepper *et al.*, 2004) and deliberately disturb the receiver, their application is considered controversial from a conservation perspective.
- Soft start/ramp-up procedure; the energy of the emitted sounds should be slowly increased thereby alerting marine life to the noise. Theoretically, this would allow animals to leave the area before they are exposed to potentially harmful levels of sound. The effectiveness of this procedure remains to be tested.

NOISE EXPOSURE CRITERION

For management purposes, a noise exposure criterion must be defined if a risk for sound-induced effects exists based on a well-founded assessment. Experiments with terrestrial animal models (Roberto *et al.*, 1985; Borg and Engstrom, 1989; Dunn *et al.*, 1991; Campo and Lataye, 1992; Hamernik *et al.*, 1993) have shown that acoustic energy, the peak value and the temporal structure of noises are important variables in determining significant acoustic effects. With increasing knowledge on the acoustic sensitivity and tolerance of

marine mammals, the proposed damage risk criteria or noise exposure criteria became increasingly reliable (UBA, 2003; Southall *et al.*, 2007). The UBA (German Federal Environment Agency) has developed a noise exposure criterion specific for the installation of offshore wind turbines by means of impact pile driving. It includes a safety range of 750 m around the source at which the sound energy should not exceed 160 dB re 1 μ Pa²·s to avoid an onset of a temporary threshold shift TTS in marine mammals, especially in harbor porpoises (UBA, 2003). The criteria recommended by Southall *et al.* (2007) comprise a matrix of levels, including maximum exposure levels for all cetacean and pinniped species for different types of acoustic signals (single pulses, multiple pulses and non-pulses). In contrast to the German noise exposure criterion, they refer to values for the onset of a permanent threshold shift (PTS) as the criterion for injury of animals and use the onset of TTS as the criterion for significant behavioral reactions. However, there are no auditory data available for some species or even higher taxonomic groups (e.g. Baleen whales) (Southall *et al.*, 2007), resulting in reduced reliability of extrapolating the data between taxa. The same applies to the sound types, as no data exist on the auditory tolerance of marine mammals e.g. for multiple exposures to intense sound (possible effects: see Hamernik *et al.*, 1987; Ahroon *et al.*, 1996), their tolerance for any other type of cumulative exposure or on PTS in marine mammals. The potential effects on marine mammals are thus difficult to assess. Therefore, any noise exposure criterion will be subject to change as soon as new relevant information becomes available independent of the national legal requirements.

OUTLOOK

One of the largest problems in studying the effects of sound on marine mammals is to find direct evidence for the cause-effect relationship between the exposure and a particular effect. Due to their relative inaccessibility, it is difficult to study effects in the wild. Exceptions are so-called controlled exposure experiments (Nowacek *et al.*, 2004; Tyack *et al.*, 2004) when the most relevant parameters can be controlled. Otherwise effects are mostly tested in animals held under human care. As long as no immediate access to a marine mammal is possible directly after an exposure to sound, any physiological or physical effect caused by the exposure cannot be reliably assessed. If sound exposures should have direct or indirect negative consequences for an individual, it may remain undetected at sea, or if, in an extreme case, an animal dies after an exposure, it may strand at a distant shore in a decomposed state long after the exposure took place. Investigations of the middle and inner ear require very fresh condition of the tissues. It is difficult to judge morphological changes of the ear and draw conclusions on their effects on stranded carcasses. The scope of noise-related effects in marine mammals is thus difficult to assess. While individual cases indicate cause-effect relationship (Parsons *et al.*, 2008), it is unclear whether protective measures at their current levels are excessive or inadequate.

However, with the increased awareness and scientific knowledge of noise-related problems for the marine environment in general and marine mammals in particular, it is likely that useful measures will be found to conduct most of the anthropogenic activities to the

same extent as is currently the case, but with fewer negative consequences. Some techniques are likely to become less “noisy” as some of the noise generated by large ships is due to cavitation caused by damaged propeller blades. This effect reduces the propulsion efficiency of the blade and increases the fuel consumption. With rising oil prices ship-owners will likely pay more attention to the quality of their ships’ blades to reduce cost, with the positive side-effect of reduced sound emissions into the water. In case this trend does not appear soon enough, environmental regulations may account for the need to reduce the contribution of shipping to the overall noise budget.

With regard to other techniques, there may be a co-evolution between the need to reduce the sound emissions (e.g. due to legislative requirements) and the intent to continue or increase the scale of a particular activity. Instead of using airgun arrays e.g. (which replaced the use of explosives a few decades ago) for marine geophysical surveys, a marine vibrational device, the so-called “vibroseis”, may become available (Weir and Dolman, 2007). This technique still relies on intense sound to probe the geological structures of the sea floor, but the sound source will be positioned near the bottom and source levels will be substantially reduced.

An alternative method for installing pile foundations for offshore wind turbines and virtually causing no sound emissions into the water column already exists. On land and especially near sensitive buildings or close to human homes, sheet piles (also tubular piles) are hydraulically pressed into the ground (e.g. GIKEN Europe, Berlin). Except for the machinery noise, no further sound emissions are linked to this method. The increased awareness for the sound-related problem may already suffice to cause efforts to reduce the effects of the installation of offshore wind turbines. If economic aspects should impede the use of such methods, there may be again the need for regulatory activity to implement such alternative methods based on environmental concerns.

McCarthy and Miller (2002) found that the noise produced by individual noise sources may have decreased and attributed this to new technologies and improved efficiency. However, the use of these activities has increased (McCarthy and Miller, 2002; Hildebrand, 2004) leading to the aforementioned increase in the overall noise level. There appears to be an upper limit to the potential of new technologies and more efficient methods to compensate for an increased level of individual anthropogenic activities.

The potential for reducing the acoustic emissions may not exist for every anthropogenic activity, or some may intentionally become more powerful (e.g. the development of Low Frequency Active Sonar; Department of the Navy, 2001; Parsons *et al.*, 2008). Those activities will become a more dominant problem with regard to noise and its effects on marine mammals. There is, however, a trend towards including issues related to noise into national and international legislation on the marine environment. Ideally, this will prevent the increase of noise in the seas from reaching levels where obvious adverse effects for a substantial number of animal species may emerge. Reducing the risk of acoustic effects from anthropogenic activities on marine fauna may be sensible even in the absence of comprehensive scientific evidence.

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GLOSSARY (DEFINITIONS AND ACRONYMS)²

- ABR:** Auditory Brainstem Response; (see “AEP” and “Auditory Evoked Potential Method” in “Introduction” for details).
- Acoustic Pressure:** The force per unit area exerted by a sound wave above and below the ambient or static equilibrium pressure is called the acoustic pressure or sound pressure. The units of pressure are pounds per square inch (psi) or, in the SI system of units, Pascals (Pa).
- Acute effect:** An adverse effect resulting from a single exposure to e.g. a substance or energy.
- AEP:** Auditory Evoked Potential; electrical changes of the nervous system that occur in response to a sound (see “Auditory Evoked Potential Method” in “Introduction” for details).
- Air bubble curtain (ABC):** A system used to reduce the sound pressure / sound energy underwater by releasing large amounts of air through a perforated tube at the bottom of the water column. Sound waves are reflected, scattered or absorbed upon hitting the surface of an air bubble due to the large impedance mismatch between water and air.
- Ambient noise:** The background noise in an area or environment being a composite of noise from many sources near and far.
- Amplitude:** The maximum positive and negative deviation of a wave, e.g. a sound wave.
- AM-signal:** Amplitude-modulated signal
- Anthropogenic:** Effects, processes, objects, energy, or materials that are derived from human activities, as opposed to those occurring naturally.
- Anthropogenic noise:** Collective for all human produced noise sources.
- Audiogram:** The sound level hearing threshold plotted as a function of frequency.
- Bft.:** Beaufort; Scale for classification of wind speed.
- Chronic effect:** An adverse effect resulting from long-term exposure to e.g. a substance or energy.
- Decibel (dB):** A logarithmic scale for describing differences in e.g. sound pressure relative to a reference pressure. The standard reference for in-air sound is 20 micro-Pascal (μPa), for underwater sound pressure 1 μPa . The dB symbol is followed by a second symbol identifying the specific reference value (i.e., re 1 μPa).

² compiled from numerous sources (e.g. Hastings, M. C., and Popper, A. N. (2005). “Effects of sound on fish. California Department of Transportation, Contract 43A0139 Task Order, 1).

- DRC: Damage risk criterion.
- EFR: Envelope following response (see “AEP” and “Auditory Evoked Potential Method” in “Introduction” for details).
- Emission vs. Immission: With regards to exposure to sound, “emission” refers to sound from the source and “immission” refers to sound received by a person or animal.
- Ensonification: The words, “insonify” and “ensonify,” are often used as synonyms but, they have subtle but different meanings. “Sonify” is a verb that simply means, “to add sound.” Likewise “insonify” means “to add sound into.”
- Fast Fourier Transform (FFT): Transforms digitised waveforms to the frequency domain.
- Frequency bandwidth: The range of frequencies over which a sound is produced or received.
- Frequency spectrum: See Spectrum.
- Hertz (Hz): The units of frequency where 1 Hertz = 1 cycle per second. The abbreviation for hertz is “Hz.”
- Immission: See Emission vs. Immission.
- Impulse: See Impulse sound.
- Impulse sound: Transient sound produced by a rapid release of energy, usually electrical, mechanical or chemical such as circuit breakers, airguns or explosives. Impulse sound has extremely short duration and extremely high peak sound pressure.
- Infrasound: Sound at frequencies below the hearing range of humans. These sounds have frequencies below about 20 Hz.
- Insonification: See “ensonification.
- MRTF: Modulation rate transfer function. A function describing the dependence of acoustic evoked EFR-amplitudes from the modulation rate of the carrier frequency.
- NIHL: Noise induced hearing loss.
- OWT: Offshore wind turbine.
- Peak pressure: The highest pressure above or below ambient that is associated with a sound wave.
- Permanent threshold shift (PTS): A permanent loss of hearing caused by some kind of acoustic trauma. PTS results in irreversible damage to the sensory hair cells of the ear, and thus a permanent loss of hearing. PTS can also be caused by drugs, pathologies and other factors.
- Pinger: Autonomous battery powered electronic device producing sound patterns in random or constant time intervals. Developed to deter cetaceans from gillnets.

Also used as reference source for hydrophone arrays or as acoustic measurements.

Propagation loss: Transmission losses of sound over distance through a medium (air, seawater). The propagation losses of sound are frequency-dependent and also depend on complex number of factors (bottom structure, sediment, etc) and are mostly irregular in coastal waters. In the far-field of a sound source the rate of decrease is proportional to the distance $1/r$. In an unbounded, homogenous medium, propagation loss will be on the order of 6 dB for every doubling of the distance.

Pulse: See Impulse sound.

Rise time: The interval of time required for a signal to go from zero, or its lowest value, to its maximum value.

Root-mean-square amplitudes (rms): These amplitudes include an averaging of the pressure wave signal over a certain time window. For sinusoidal signals, the rms pressure is usually about 9 dB lower than the peak-to-peak pressure.

Shock wave: A propagating sound wave that contains a discontinuity in pressure, density, or particle velocity.

Sound attenuation: Reduction of the level of sound pressure. Sound attenuation occurs naturally as a wave travels in a fluid or solid through dissipative processes (e.g., friction) that convert mechanical energy into thermal energy and chemical energy.

Sound exposure level (SEL): The constant sound level acting for one second, which has the same amount of acoustic energy, as indicated by the square of the sound pressure, as the original sound. It is the time-integrated, sound-pressure-squared level. SEL is typically used to compare transient sound events having different time durations, pressure levels, and temporal characteristics. It is given dB re $1 \mu\text{Pa}^2\cdot\text{s}$.

Sound Pressure Level: Pressure level of a sound source measured at a certain distance from a sound source and commonly referred to a reference pressure level of $1 \mu\text{Pa}$ and expressed in dB re $1 \mu\text{Pa}$.

Source level (SL): is the Sound Pressure Level of a sound source measured on the acoustic axis at a distance of 1 m from the source. In underwater acoustics this level is commonly referred to a reference pressure of $1 \mu\text{Pa}$. The definition is $10 \log$ intensity, divided by the reference intensity and expressed in dB (decibel) re (relative to) $1 \mu\text{Pa}$ at 1 m.

Spectrogram: A graph, which displays acoustic energy as a function of frequency allowing frequency patterns to be visualised, and reverberations to be depicted.

Spectrum: A graphical display of the contribution of each frequency component contained in a sound.

SPL: Sound pressure level

Temporary threshold shift (TTS): Temporary loss of hearing as a result of exposure to sound over time. Exposure to high levels of sound over relatively short time periods will cause the same amount of TTS as exposure to lower levels of sound over longer time periods. The mechanisms underlying TTS are not well understood, but there may be some temporary damage to the sensory hair cells. The duration of TTS varies depending on the nature of the stimulus, but there is generally recovery of full hearing over time (defined as period of 30 days, otherwise considered PTS).

Threshold: The threshold generally represents the lowest signal level an animal will detect in some statistically predetermined percent of presentations of a signal.

Transducer: A device (hydrophone e.g.) to convert underwater sound into electrical voltage.

TS Threshold Shift

Ultrasound: Sound at frequencies above the hearing range of humans. These sounds have frequencies above about 20 Hz.

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DESCRIPTION OF THE INDIVIDUAL SCIENTIFIC CONTRIBUTIONS TO THE MULTIPLE-AUTHOR MANUSCRIPTS

Chapters 1-5 included in this thesis were written as manuscripts with multiple authorship. In the following, the contribution of each co-author to the individual papers is described.

CHAPTER 1: DEVELOPMENT AND TESTING OF A RAPID METHOD FOR AUDITORY SCREENING IN HARBOR PORPOISES (*PHOCOENA PHOCOENA*)

Authors: Klaus Lucke^{1*}, Eligius Everaarts², Bert Hoeve², Niels van Elk², Mirco KÜchler¹, Ursula Siebert¹

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Klaus Lucke developed the idea for the study, conducted the auditory measurements and data analysis. Ursula Siebert supervised the study. Eligius Everaarts and Bert Hoeve supported the auditory measurements logistically. Niels van Elk was responsible for the health check prior to and supervised the health monitoring during the measurements. Mirco KÜchler assisted Klaus Lucke in some of the auditory measurements and the data analysis. The statistical analysis was conducted with support from Sven Adler. The manuscript was written by Klaus Lucke and reviewed by all co-authors.

CHAPTER 2: IN-AIR HEARING SENSITIVITY IN HARBOR SEALS (*PHOCA VITULINA*) FROM THE NORTH SEA AS A BASELINE FOR LONG-TERM AUDITORY MONITORING

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Klaus Lucke developed the idea and concept for the study, conducted the measurements and data analysis. Janne Sundermeyer assisted Klaus Lucke in the measurements and data analysis. She also conducted complementary AEP measurements and analyzed these data in her diploma thesis. Jörg Driver was the responsible for the health check and monitoring and provided important input with regard to the immobilization. Tanja Rosenberger is responsible for the well-being of the harbor seal pups, and assisted Klaus Lucke in the AEP measurements. Ursula Siebert supervised the study. The manuscript was written by Klaus Lucke and reviewed by all co-authors.

CHAPTER 3: PERCEPTION OF LOW-FREQUENCY ACOUSTIC SIGNALS BY A HARBOR PORPOISE (*PHOCOENA PHOCOENA*) IN THE PRESENCE OF SIMULATED OFFSHORE WIND TURBINE NOISE

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Klaus Lucke and Niels van Elk developed the idea for the study. All auditory measurements were conducted and analyzed by Klaus Lucke. Paul Lepper supported Klaus Lucke with the stimulus generation and recording and conducted the analysis of the acoustical data. Eligius Everaarts and Bert Hoeve trained the animal and conducted the behavioral procedures with the animal during the measurements. Niels van Elk was responsible for the health check prior to and supervised the health monitoring during the measurements. Ursula Siebert supervised the study. The statistical analysis was conducted by Klaus Lucke with support from Roger Mundry. The manuscript was written by Klaus Lucke and reviewed by all co-authors.

CHAPTER 4: TEMPORARY SHIFT IN MASKED HEARING THRESHOLDS IN A HARBOR PORPOISE (*PHOCOENA PHOCOENA*) AFTER EXPOSURE TO SEISMIC AIRGUN STIMULI

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The idea and concept for this study were developed by Klaus Lucke. He conducted the AEP measurements and analyzed all auditory data. Paul Lepper supported Klaus Lucke with the stimulus generation as well as the recording and analysis of the acoustical data. Marie-Anne Blanchet trained the animal and conducted the behavioral procedures with the animal during the measurements. The manuscript was written by Klaus Lucke and reviewed by all co-authors.

CHAPTER 5: THE USE OF AN AIR-BUBBLE CURTAIN TO REDUCE THE RECEIVED SOUND LEVELS FOR HARBOR PORPOISES NEAR AN IMPULSIVE SOUND SOURCE

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The idea for this study was developed by Klaus Lucke. The system was designed and constructed by Klaus Lucke and Paul Lepper. They conducted the acoustic measurements and analysis. The concept for the behavioral study was designed and analyzed by Klaus Lucke and Marie-Anne Blanchet with support from Roger Mundry. Marie-Anne Blanchet conducted and supervised the behavioral observations. The manuscript was written by Klaus Lucke and Paul Lepper and reviewed by all co-authors.

ERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Arbeit selbständig von mir angefertigt wurde und dass sie, abgesehen von der Beratung durch meinen Betreuer, nach Inhalt und Form meine eigene Arbeit ist. Sie wurde keiner anderen Stelle ganz oder zum Teil im Rahmen eines Prüfungsverfahrens vorgelegt. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis entstanden. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Biologische Meereskunde erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

Kiel,.....

Klaus Lucke