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TTS growth and recovery in harbor porpoises exposed to intermittent and continuous signals

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Executive Summary

This report describes the results of two separate IOGP JIP-funded projects (I) and (II), both aimed at studying Temporary Threshold Shift (TTS) in harbor porpoises (*Phocoena phocoena*) exposed to repeated airgun exposures. The objective of this study was to obtain data to develop a scientifically valid method to predict TTS growth, recovery, and affected hearing frequencies as a function of cumulative sound exposure level, taking into account the number of airgun pulses and shot time interval.

Two porpoises were available at the SEAMARCO Research Institute, based in the Netherlands. One animal was successfully trained to participate in two TTS studies with airguns using a behavioral protocol to measure TTS. During these experiments the porpoise was exposed to intermittent exposures of a compact array of 1 to 4 scaled-down airgun(s). The number of airgun shots ranged between 1 and 1350, with exposure durations up to 90 minutes. In total, 174 (Project I) and 72 (Project II) exposure sessions (including TTS sessions, control sessions and training sessions) were carried out over a period of approximately 1.5 years. The two projects combined resulted in one peer-reviewed publication (Kastelein et al., 2017b); the submission of two manuscripts to a peer-reviewed journal (Kastelein et al., 2019c; Kastelein et al., close to submission to JASA); and a fourth manuscript in preparation (von Benda-Beckmann et al., in prep).

The main finding from study (I) was that only low levels of TTS_{1-4} (~ 5 dB) could be achieved with airgun exposures resulting in cumulative sound exposure level (SELCum) up to 191 dB re 1 µPa²s (published in Kastelein et al., 2017b). These SELCum were 11 - 26 dB higher than those achieved in previous experiments by Lucke et al. (2009) and Kastelein et al. (2015a) measuring TTS in porpoises exposed to impulsive sound. It was found that the highest TTS was observed for frequencies of 4-8 kHz, even though the one-third-octave (base 10) band SEL for lower frequencies (500 Hz) was 18 dB higher. A comparative analysis of the different exposure experiments showed that frequency-weighted SELCum (Very High Frequency cetacean weighting following Southall et al., 2019) was a good
predictor of the amount of TTS, as well as the frequency range in which TTS occurred. This supported the concept of frequency weighted SELcum as a predictor for hearing loss as has been proposed recently (Finneran, 2016; NMFS, 2018; Southall et al., 2019).

In the second study (II), it was found that TTS onset reported in Kastelein et al. (2017b) could not be reproduced, even though the same animal was exposed to higher SELcum (up to 199 dB re dB re 1 µPa²s, i.e. 8 dB higher than during Project I). Analysis of the data and exposure conditions remain inconclusive because of limited data but suggest that the animal may have learned over time to self-mitigate against the repeated impulse exposures (Kastelein et al., submitted).

In addition, it was found that peak sound pressure alone was not a good predictor for TTS. The highest peak sound pressure levels for a single airgun exposure achieved in this study (Lp,pk = 199 dB re 1 µPa²s; Kastelein et al., 2017b; Lp,pk = 202 dB re 1 µPa²s in Kastelein, submitted) was comparable to those observed in Lucke et al. (2009). However, single exposures did not generate any significant TTS, and TTS was only observed after at least 10 shots. The highest peak sound pressures observed in experiments I and II are at a similar level with the current NMFS (2018) and Southall et al. (2019) threshold for predicting the risk of a permanent threshold shift (PTS) in harbor porpoises. Nevertheless, TTS could not be elicited in experiment II at these levels.

Both studies relied on a porpoise that was trained to participate in a TTS experiment. The second study was terminated prematurely because of the illness of this animal. Unexpectedly, and despite treatment with antibiotics, the animal died of pneumonia (caused by methicillin-resistant Staphylococcus aureus bacteria (MSSA)) after showing signs of illness (reduced food intake) for only two days before his death. As discussed in detail in the report, repeated tests of hearing before and during the experiments that were conducted showed no diminished nor abnormal hearing prior to the onset of the illness. Therefore the data were reliable albeit limited. Unfortunately, the loss of the animal precluded further testing of the hypothesis of self-mitigation or further experiments with increasing SELcum in order to study a wider range of exposure conditions, and effective quiet levels.

A second animal could not be trained to cooperate in the TTS experiments, because it showed a stronger behavioral response to the airgun exposures than the other animal. The introduction of a bubble-plastic screen between the exposure pools was successful in reducing the high-frequency components of the airgun signal in the indoor pool where the second animal was kept, allowing for the TTS experiments with the first animal to be carried out in the outdoor pool. Qualitative observations of the animals' behavior in the indoor pool suggested that high-frequency content of the airgun sound was responsible for triggering the response in both animals (Kastelein et al., 2019c).

This report reviews existing published data on marine mammal, human, and laboratory animal temporary hearing loss growth and recovery functions and provides an overview of the mechanisms underlying TTS and PTS. Based on this literature review, two different approaches for predicting TTS were considered: the modified-power law (MPL) model, and a kurtosis-corrected equal energy model.
An adapted version of the MPL model is introduced in this report, which attempts to overcome some shortcomings of the original model, which required growth and recovery from individual exposures to be measurable (Humes and Jesteadt, 1991; Finneran et al., 2010; Finneran, 2015a). This model was evaluated using TTS growth data obtained for harbor porpoises. Because of the limited number of exposures resulting in TTS due to airgun sounds obtained in this study, previous exposures using intermittent and continuous 1 – 2 kHz active sonar exposures (Kastelein et al., 2014a; 2015a) were used to evaluate and discuss the potential and limitations of the proposed models instead. Fitting of the data resulted in parameters that could explain TTS growth observed in a wide range of sonar exposure conditions (SPLs, 10% duty cycle vs continuous exposure), but underestimated the TTS growth of exposure to impulse sound (airguns and pile driving) and sonar with higher (6-7 kHz) frequencies. This suggests that the approach may be promising, but full validation requires further model development and added data of TTS growth due to different signal parameters of the fatiguing sound.

The kurtosis correction to frequency weighted SEL, which has been proposed for humans and terrestrial mammals, led to more consistent TTS onset thresholds, especially when the free parameter in this model was tuned specifically to the harbor porpoise TTS growth data. However, the model fails to account for recovery between pulses. It was concluded that an adapted version of the modified-power law (MPL) model (Humes and Jesteadt, 1991) was the most promising model for TTS growth that allowed for integrating the effect of hearing recovery between intermittent airgun shots.

Recommendations are provided for future experiments aimed at understanding the hearing recovery between multiple exposures and airgun levels corresponding to effective quiet.
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Abbreviations

PTS  Permanent Threshold Shift
TTS  Temporary Threshold Shift
TTS$_t$ Temporary Threshold Shift measured $t$ minutes after the end of the exposure session
CTS  Compound threshold shift; amount of hearing threshold shift observed which may be combination of TTS and PTS
ATS  Asymptotic threshold shift - TTS growth reaches a plateau with increasing exposure duration, beyond which no further TTS occurs
AEP  Auditory evoked potentials
Cochlear toughening A reduction in TTS during a prolonged exposure, indicating that the hearing system is desensitized to the exposure signal.
IHC  Inner Hair Cell, a component of the inner ear related primarily to transduction of sound energy into neural impulses.
OHC  Outer Hair Cell, a component of the inner ear related primarily to amplification and frequency discrimination
Stereocilia The mechanosensing organelles of hair cells, which respond to fluid motion in numerous types of animals for various functions, including hearing ability
Afferent neurons Neurons that transmit signal detected in the IHCs
Efferent neurons Neurons that transmit signal from the brain to IHCs and OHCs, reflex associated with modifying reception of sound energy. Part of the acoustic reflex
Excitotoxicity The pathological process by which nerve cells are damaged or killed by excessive stimulation by neurotransmitters
Effective quiet An SPL which does not contribute to the growth of TTS, or delay the recovery of TTS
Stapedial reflex An acoustically mediated response in the middle ear, which leads to contraction of the stapedial muscle. This allows for less energy to be transmitted to the inner ear
SEL$_{ss}$ ($L_{p,E,ss}$) Single shot/signal sound exposure level
SEL$_{cum}$ ($L_{p,E,cum}$) Cumulative sound exposure level of multiple exposures
SPL ($L_p$) Sound Pressure Level
$L_{p,pk}$ Peak sound pressure level (zero-to-peak)
MPL model Modified-Power-Law – model used for summing TTS over multiple exposures
1 Introduction

1.1 Background

The oil and gas industry regularly carries out seismic surveys for exploration purposes. The seismic surveys use acoustical sensing, with airguns as the primary acoustic source; therefore, the sound radiated by airguns is coming under increasing regulatory scrutiny. Marine mammals are considered to be sensitive to anthropogenic sounds, both auditory and behaviorally. Airguns used in seismic surveys produce intense broadband impulsive sound which may cause harmful auditory effects in these animals, in the vicinity of the airgun array.

The harbor porpoise (Phocoena phocoena) is known to be especially sensitive to sound. For that reason, this species is used by regulators as an “indicator species”, and its onset of TTS is used both to estimate injury thresholds (PTS) based on the TTS-SEL growth rate (e.g. Southall et al., 2007, 2019; NMFS, 2018) and as a risk threshold in its own right (BMU, 2013).

Little is known about the growth of TTS in porpoises exposed to impulsive sound with increasing dose of exposure, which is often expressed in terms of sound exposure level (SEL). The increase in TTS with increasing dose (the ‘growth rate’) is important for estimating permanent hearing threshold shift (PTS) onset levels. The basis for current PTS onset thresholds is poor, as it relies on a (precautionary) growth rate based on measurements of TTS growth in terrestrial mammals. These values are used to extrapolate from relatively low levels of TTS (~6 dB) to high levels of TTS (~40 dB) that are believed to result in PTS (see Southall et al., 2019, and references therein). An experimental characterization of the growth rate in harbor porpoises should result in a more accurate extrapolation over a smaller amount of TTS, which would lead to a firmer scientific basis for PTS onset level estimations for airgun sounds.

Criteria for TTS onset due to exposure to multiple impulsive pulses in current use to assess the impact of seismic surveys are based on the cumulative exposure (SELcum) over all shots, without taking into account recovery of hearing between successive impulses. Available evidence for navy sonar (Kastelein et al., 2014a, 2015a) suggests that the SELcum required to cause a 6 dB temporary threshold shift (TTS onset) depends on SPL, duty cycle, and exposure duration. Recent TTS studies with harbor porpoises by SEAMARCO for pile driving sounds (inter-pulse interval 1.3 s) have shown that TTS growth due to intermittent sounds is much slower than for continuous sounds, presumably due to recovery of hearing between pulses. Because the inter-pulse interval during a seismic survey is typically in the order of 10 seconds, we also expect hearing to recover between airgun pulses.

Current measurements of hearing threshold shifts in porpoises exposed to airgun sound were done at frequencies that are much higher than the frequencies dominating the acoustic energy content of the airgun signal. The frequency range of an animal’s hearing affected by TTS when exposed to broadband impulsive sound, and how fast hearing recovers are not known. An extended duration of reduction in hearing sensitivity in a frequency range of importance to an animal’s ecology might have serious long-term consequences for its fitness. On the other hand, if TTS or
PTS is caused in hearing frequencies that are of little ecological significance, the impact on an animal's fitness may be less severe or less relevant than other environmental factors. Recent studies of porpoises exposed to naval sonar sounds, show that the frequency of maximum TTS can occur beyond the frequency range of the stimuli with increasing SEL (Kastelein et al., 2014b). Therefore, by measuring the frequency range affected and the recovery times, we investigated how the frequency content of the exposure signal relates to frequencies at which TTS occurs.

1.2 Research Objectives and Outline

The objective of this study was to obtain data and develop a method to predict TTS growth, recovery, and frequency specificity, as a function of the cumulative sound exposure, taking into account the number of airgun pulses and the inter shot interval. To motivate the underlying models, relevant publications of TTS growth and recovery (due to impulsive sound and sweeps) were reviewed in order to identify data gaps for carrying out new measurements targeted at filling these gaps and for developing predictive models.

This report reviews existing published data on both marine mammal and human/lab animal hearing loss growth and recovery functions and provides an overview of the mechanisms underlying TTS and PTS (Chapter 2). We describe the data collection of TTS growth and recovery in a harbor porpoise exposed to airgun sounds (Chapter 3). Based on the review, we selected promising methods, which then were evaluated against data on TTS growth due to intermittent sound and continuous sound exposures in harbor porpoises (Chapter 4).
2 Hearing loss and recovery due to impulsive and intermittent sound exposure in terrestrial and marine mammals

The ability or sensitivity to hear a sound is typically expressed in terms of the hearing threshold or the level of sound required to detect each frequency within the hearing range of a species. Thresholds can be measured using a number of different techniques: behavioural, AEP, or otoacoustic. When the hearing system is over-exposed to sound, a raising of the hearing threshold is observed, which is called a threshold shift (TS). A threshold shift that recovers to the baseline hearing threshold after some duration is called a ‘temporary threshold shift’ (TTS) and is called ‘permanent threshold shift’ (PTS) when it does not fully recover.

Hearing ranges vary among species, and even when two species may hear the same frequencies, they can have gross differences in their sensitivities to each frequency. These differences are the result of differences in the structures of their ears and caused during evolution by differences in the species ecology.

2.1 Basic functioning of a mammalian hearing and measuring threshold shifts

The basic mammalian hearing system consists of three components: the outer ear, the middle ear and the inner ear.

The outer ear acts principally as a high efficiency (low impedance) sound collection system. In land mammals, the outer ear consists of an external “pinna” on the sides of the head and a canal that “funnel” air borne sound to the middle ear. In cetaceans, there is no external ear but rather a set of shaped fatty tissues found along the lower jaw that are matched to the impedance of water and similarly collect and direct sound to the middle ear.

Sound is amplified by the middle ear structures and transmitted to the inner ear. The functional parts of the mammalian middle ear consist of the eardrum, three connected bones (the ossicular chain: malleus, incus, and stapes), and two muscles, the tensor tympani and stapedial muscles and their associated ligaments and tendons, that affect the motion of these bones (Fig. 2.1). Activity of these bones and their control that may be related to diminishing sound impacts is discussed further in the following section on self-suppression of hearing sensitivity.

The ossicular chain ends in the stapes, which is attached to the spiral, inner ear labyrinth (cochlea) at the oval window. Pulsations at the oval window are transmitted throughout the cochlea, resulting in motions of the basilar membrane (BM) (Figure 2.1). The basilar membrane is a tuned structure, meaning it responds differentially along its length to incoming frequencies. The range of response frequencies and degree of response varies with species. When parts of the BM move, hair cells located at these points respond with a release of chemicals that produce electrical signals in attached neurons that are sent to the auditory centers of the brain.
Hair cells can be divided into inner hair cells (IHC), which have primarily “afferent” neurons that are responsible for transmission of acoustic signal information to the brain. The outer hair cells (OHC) have primarily “efferent” neuron connections that conduct control responses coming from the brain. The efferent signals affect the OHC shape that changes the OHC ability to respond. In some cases the brain’s feedback signals to OHC can sharpen the response to sound or in others it can decrease sensitivity to sounds (Robles and Ruggero, 2001).

Impacts from sound may differ according to whether IHCs and OHCs or both are affected. Damage to IHC results generally in a loss of ability to respond to frequencies normally detected at that location, whereas damage to the OHCs increases the thresholds for response; i.e., the frequency may be detected but only at higher sound levels. Efferent neurons also play a role in self-mitigation in that the brain will in some cases, such as during continuous sound saturating the afferents, or in response to a stapedial reflex, send a signal that shuts down hair cell response, although this usually operates only for very short times, on the order of milliseconds.

2.2 TTS growth studies in human and terrestrial animals

TTS growth and recovery have been reported for a wide variety of exposure conditions and species. A number of papers provide extensive reviews on these studies (e.g. Melnick, 1991, Finneran, 2015a). TTS growth typically follows an exponential growth, possibly followed by a plateau when the TTS reaches the so-called asymptotic threshold shift (ATS; a maximum induced TTS that does not increase even when exposure duration is increased, Melnick, 1991). In some instances a reduction in TS during the exposure (cochlear toughening) can be observed (Hamernik et al., 2003). The exposure level associated with the turning point where the TTS grows from logarithmically with SEL to a linear relation is referred to as the ‘critical level’ and has been argued to constitute a point at which mechanical fatiguing processes start to dominate over metabolic processes (Price, 1981; Hamernik et al., 1987). For humans, TTS growth and recovery can be predicted well as percentage of the ATS induced by the exposure (Melnick, 1991). However, ATS has not been observed in marine mammals, which may be due to avoiding the long exposure durations employed in land mammal laboratory studies required to reach an ATS such as those on chinchillas and primates (4-36 h) (Bohne and Clark, 1982; Clark, 1991; Yoshida and Liberman, 2000; Finneran, 2015a). Also, in addition to differences in exposures, the target level of TTS (often greater than 20 dB shifts) and “acceptable” recovery times (as long as 30+ days) common in land mammal data are not attempted with marine mammals. Further, even in laboratory studies, the results vary among species and across experimental paradigms (Yoshida and Liberman, 2000; Décory et al., 1990) even with equivalent exposures.
Figure 2.1 Panel A: Harbour porpoise inner ear/cochlea (left and center) and middle ear (right) structures. Panel B: Schematic zoom-in on a cross-section of the major structures of the organ of Corti showing the inner and outer hair cells (IHC resp. OHC) with their stereocilia, situated on top the basilar membrane, and afferent/efferent neuron fibers with synapses at the base of hair cells. Image credits and species shown: Panel A: Ketten (porpoise); Panel B: adapted from Kujawa and Liberman (2009) (mouse).

In laboratory studies over the last 50 years, TTS studies have commonly employed exposures of 15 mins or longer, often for multiple hours, at SPLs over 100 dBA re 20 µPa, eliciting shifts of 40-50 dB with recoveries considered as acceptable after 2 or more days (Miller et al., 1963) and in some cases up to one month post exposure. These studies typically induce larger initial TTSs than are reported for marine mammals and, similarly, have complete recovery times that are longer for these larger shifts. It is also noted in Price and Kalb (2018) that at the very highest intensities recovery is delayed and often results in PTS (Hamernik et al., 1987; Luz and Hodge 1971; Price 1983).
Key points of consensus that have emerged across experiments in multiple mammal species about the nature of threshold shift risk are as follows:

**Damage Risk**
- Damage is roughly proportional to total sound energy (duration \( \times \) intensity).
- Intermittent exposures are less dangerous than continuous.
- Low frequencies are generally less dangerous than higher frequencies.
- Narrow-band sounds are more dangerous than broad-band.
- Large inter-subject variability in noise resistance (susceptibility to TTS/PTS).

**Growth, Recovery & Pattern**
- Hearing loss deteriorates to asymptote during continued exposure.
- Recovery begins after termination of the exposure.
- Above "critical level", hearing loss grows rapidly.
- Hearing can improve over long periods (up to 1 month post-exposure, possibly even longer).
- Narrow-band exposure: maximum loss near exposure center frequency at lower SPL.
- The frequency with maximum hearing loss shifts up about 1/2 octave with increasing SPL.
- Broad-band exposure, maximum loss occurs at mid frequencies within the hearing range.

### 2.3 Mechanisms for hearing loss and recovery due to impulsive sound exposure in land mammals and marine mammals

Mechanisms for TTS and PTS have been reviewed in several studies (Nordmann et al., 2000; Heinrich et al., 2006; Ryan et al.; 2016; Kurabi et al., 2017; Liberman et al., 2017), which indicate several key observations:
- Different mechanisms underlie TTS and PTS.
- Different mechanisms may mediate TTS.
- Hair cells of the organ of Corti are the most vulnerable to permanent noise damage.
- Most PTS (>50 dB loss) is due to damage to the stereocilia of the hair cells.
- Severe TTS causes little overt pathology: changes are sub-microscopic.
- IHC damage results in loss of transduction; OHC damage raises thresholds.
- Hair cell loss leads to retrograde neuronal death over time.

Clearly, mechanisms underlying TTS must be to some extent recoverable. Many of these are still being investigated. The majority of the mechanisms that are considered to be related are fatigue, swelling, buckling, or recovery of structures that are sub-microscopic, such as the fine hair-like projections atop the hair cells (stereocilia, Fig. 2.1) and strands between them (tip-links) (Heinrich and Feltens, 2006; Liberman et al., 2017, Ryan et al., 2017).
Figure 2.2 Schematic summary of underlying mechanisms causing noise-induced TTS and PTS and their dependencies on the level of the noise and exposure duration, and middle ear and inner ear mechanisms that mediate hearing sensitivity of animals.

**Cochlear noise-induced hearing loss mechanisms**

- **Effective quiet**
  - (reversible) activation of ion channels by extracellular ATP
  - (reversible) excitotoxicity to cochlear afferent neurons
  - (reversible) OHC clip: link breakage

- **Critical level**
  - (non-reversible) excitotoxicity to cochlear afferent neurons

- **Permanent loss of HC**
  - (non-reversible) Mechanical disruption of HC stereociliary arrays
  - (non-reversible) Disruption of IHCs and supporting cells

- ** PTS**
  - (non-reversible) exposure of the basal poles of remaining intact IHCs to high levels of potassium, leading to HC death

- **Blast exposure**

**Chronic exposure**
- (metabolic fatigue)

**Permanent loss of afferent neurons in HC**
- Step 1: Degeneration of unmyelinated terminal dendrites of SGCs
- Step 2: The loss of these peripheral terminals interrupts the neurotrophin signaling (required for normal development and maintenance of the cochlear innervation)
- Step 3: interruption of neurotrophin signaling compromises the long-term viability of those neurons

**Cochlear toughening?**

**Middle-ear mechanisms contributing to reduced sensitivity to impulsive and loud noise exposures**
- Middle ear muscle response (stapedial reflex)
- Peak-clipping due to the limited dynamic range in displacement of the annular ligament of the stapes

**Inner ear and neural mechanisms involving efferent inhibitory projections mechanisms contributing to reduced sensitivity to impulsive and loud noise exposures**
- Acoustic reflex by decreasing the gain of the OHC cochlear amplifier
- Acoustic reflex by increasing the resistance against excitotoxicity in the IHC
Mechanisms that lead to permanent damage to hair cells, or their afferent neurons, which can produce permanent threshold shift (PTS), are likely mediated by biochemical processes that occur within the cells themselves (Liberman and Kujawa, 2017; Kurabi et al., 2017). One recent hypothesis for noise-induced permanent hearing loss is that an excess of glutamate in the system produces “excitotoxicity” and an imbalance of calcium ions that are fundamental to degeneration of inn ear structures observed after noise exposure (Kujawa and Liberman, 2009; Liberman and Kujawa, 2017).

In the case of blast exposures, in addition to the potential for immediate mechanical destruction of some ear structures (dislocation of ossicles, blow-out of inner ear membranes), the exceptional peak levels of the associated sound wave, can produce acute damage to inner ear components at a greater distance from the sources, such as disruption of HCs, stereociliary arrays, and supporting cells, with collateral damage to adjacent cells from the flood of intracellular fluids (Liberman and Beil, 1979; Slepecky, 1986; Patuzzi et al., 1989; Kurabi et al., 2017), These processes all result in a permanent threshold shift.

To summarize, Figure 2.1 represents the key structures that are involved in the occurrence and mitigation of TTS. Mechanisms underlying TTS can affect both the structure and integrity of the inner ear, producing changes in hair cell responsivity, possibly through alterations of the stereocilia (e.g., broken tip-links), through intracellular chemical changes affecting synaptic transmissions at the base of hair cells and alterations in hair cell conformation with swelling and degeneration of synaptic ribbons that transmit electrical signals from the cochlea to the brain.

2.3.1 Self-suppression of hearing sensitivity

Several recent papers (Nachtigall and Supin, 2013, 2014; Nachtigall et al 2018; Finneran et al 2015a, b; Finneran 2018) have reported the ability of trained, captive odontocetes of several species to reduce their auditory brain stem response to loud sounds. These studies have documented the “self-mitigation” response in these species but were not geared to identifying explicit mechanisms resulting in the onset and control involved in diminishing the auditory response.

Any one or a combination of mechanisms, simultaneously or sequentially, are feasible for marine mammals based upon our current knowledge of their highly developed auditory structures that contain all of the components known to be involved in reducing sensitivity to sound in other mammals. It is certainly possible that the ability to limit responsivity occurs at the central nervous system level within the auditory cortex as has been demonstrated previously for some mammals or through efferent control of hair cell activity reducing hearing sensitivity, within the inner ear as discussed above. These central and inner ear controls are well established in several vertebrate species, including cats, bats, and humans (Galambos, 1956; Harris and Dallos, 1979; Guinan, 2006). However, there are also clearly demonstrated peripheral mechanisms, such as the middle ear reflexes (also referred to as the acoustic or stapedial reflex) found in most mammals (Henson, 1965; Hung and Dallos, 1972), that may be involved as well in cetacean control of auditory responses.

Concerning what mechanisms are at work in odontocetes, we do not as yet have any evidence, much less quantification, of efferent fibers in any cetacean, but it is
reasonable to speculate that their substantial neural population (Ketten, 2000) contains both afferent and efferent elements. Similarly, they have a highly developed auditory cortex, although we do not have any direct studies of the functional or tonotopic response mapping of the central structures.

In the studies of odontocetes noted above, the authors speculated that the mechanism involved was likely to be centrally mediated, but they did not consider the possibility of it being initiated or further mediated by a peripheral mechanism as well. In all land mammals investigated to date, the middle ear acoustic reflex is a rapid, automatic reflex arc that results in a simultaneous bilateral suppression of middle ear operation in transmission of sound to the inner ear by depressing the action of the middle ear bones. As described in the previous section, the middle ear houses a series of bones that act as levers to amplify received sounds. Associated with them are muscles that can control their ability to respond. Upon exposure to an intense sound, there can be an involuntary contraction of middle ear muscles: the stapedial muscle decouples the stapes from the oval window while the tensor tympani muscle pulls on the malleus tensing the eardrum, effectively reducing the ability of the eardrum to move and decreasing the ability of the middle ear bones to transfer sound to the inner ear. The response pathways that are activated in this reflex involve the facial nerve and auditory nerve and at higher levels, the brainstem regions of the superior olivary complex and ventral cochlear nucleus (Møller, 2000). The central nervous system is also involved at the level of the auditory cortex but is not the initial response modifier. The auditory cortex is secondary and typically is an habituation or learned response. Rather, the middle ear reflex is an immediate response, independent of the slower but potentially more lasting control through the central auditory system. As a reflex arc, it involves only the activation of the ligaments and muscles in the middle ear through their peripheral neural inputs.

It is likely that both of these mechanisms are functional in marine mammals, not just the central nor peripheral path. However, in the wild, the presence of an acoustic reflex is the critical issue, considering the less likely chance of training the response. For this reason, it is important to have a better understanding of the middle ear reflex and its potential effect in marine mammals.

In humans, only the stapedial muscle is activated, but it alone reduces sound intensities reaching the inner ear by approximately 15-20 dB. In other mammals that employ both the stapedial and tensor tympani muscles, the contractions result in an effective decoupling of the whole ossicular chain, as well as increased tension on the tympanic membrane, resulting in a decrease of energy being transmitted to the inner ear; less force is applied at the oval window through the stapes, with a reduction of as much as 36 dB. The time course for initiation of this action can range anywhere from 5 to 25 ms with a curve that follows that of the sound to peak intensity. The reflex can last for multiple seconds and can be sequentially re-initiated by sounds with short intervals.

It is especially important to consider evidence for stapedial reflex in another echolocator, the microchiropteran bats. Bats actively employ a stapedial reflex as part of their suppression of hearing their out-going echolocation signal so that it does not confound reception of the echoes. Therefore, it is not surprising that odontocetes may have a similar system and perhaps strong stapedial reflex as well.
Stapedial or acoustic reflexes are well established for terrestrial mammals, but have not been demonstrated nor disproven in marine mammals. All the physical components needed for such a mechanism are present in porpoises as well as other cetaceans (Ketten, 2000; Popper and Ketten, 2008). The results in Study II invite speculation that the study animal may have employed a self-mitigation mechanism involving short and/or long term self-mitigation processes. We have been able to determine through dissection of ears and examination of histology slides that not only odontocetes (toothed whales) but also mysticetes (baleen whales) have substantial stapedial and tensor tympani muscles present in the middle ear (Fig. 2.3). Therefore, there is evidence suggesting that all cetaceans may be capable of a rapid middle ear reflex that can be further explored experimentally on captive animals and through model simulations.

It is also important to note that the level of protection from this reflex is limited and variable across individuals. While the presence of the fundamental structures is encouraging that they may have an acoustic reflex that can provide self-mitigation of loud sounds, presence alone is insufficient to assure, much less quantify, an acoustic reflex in each species. However, considering that the latency in most mammals is 8-10 ms and reaches a peak over 100 ms or more, with increasing contraction according to the stimulus level (Brask, 1978; Dancer, 1991; Møller, 2000; Jones et al, 2018) there is the potential for sufficient diminishment of auditory response to protect even naive animals in the wild from a short term, temporary exposure.

2.3.2 Effective quiet

The concept of effective quiet; i.e., sound levels low enough not to cause or contribute to temporary hearing loss, has been known for several decades and has been investigated primarily in humans (Ward et al., 1976). Due to the lack of data on effective quiet, current models tend to accumulate over all exposures over a long period (e.g., 24 h), which even for low levels may result in an unrealistic high accumulated exposure. Effective quiet has not been measured in marine mammals (Finneran, 2015).

Two different definitions of effective quiet are possible (Ward et al., 1976):
(Definition 1) Effective quiet = “The minimum SPL at which no TTS is induced, regardless of duration”.

This definition was cast in terms of a continuous noise exposure with a constant SPL. Here we re-cast that definition in terms of SELss of a single airgun exposure: “For a given duty cycle there exists an “effective quiet SELss”, below which there is no TTS onset, irrespective of SELcum (i.e. irrespective of duration and number of shots)”.

(Definition 2) Effective quiet = “the level of sound that just fails to retard recovery from TTS.”

The second definition is a more practical measure, as exposure durations with human (or animal) subjects cannot be carried out for much longer durations than 2 to 8 hours (one working day). There is no obvious reason that this need be the same as that level that just fails to produce TTS, but studies so far suggest that the effective quiet levels are of similar magnitude (Ward et al., 1976).

One of the original objectives of this study was to determine the levels for effective quiet in harbour porpoises, in order to inform exposure models that predict the risk of hearing effects due to airgun sounds. Unfortunately, due to the unexpected death of the animal trained for the airgun exposure studies, these studies could not be carried out. Appendix A reflects the considerations behind the experimental design, which will be useful for future studies trying to measure effective-quiet with different animals, or species.

2.4 Models for TS growth of intermittent and impulsive sound exposures

2.4.1 Equal energy and frequency weighting

In a recent review of TTS studies on marine mammals, Finneran (2015a) summarizes the models for predicting the TTS growth of intermittent sound exposures and how these apply to marine mammals. The review discriminates between two types of models: “exposure summation” methods and “TTS summation” methods.

The most common exposure summation method used is the “equal energy”-rule in which equal sound exposures are considered to have equal TTS growth, which is solely dependent on the total (frequency-weighted) sound exposure level, regardless of how it is distributed over time, nor does it account for potential recovery between exposures. An equal energy rule is currently used widely to predict noise induced hearing loss for humans (NIOSH, 1998) and has also been considered for marine mammal exposure criteria (Southall et al., 2007; Finneran, 2016; Southall et al., 2019). In these cases, to account for differences in the frequency sensitivity of marine species, a frequency weighting is applied to the sound exposure level, which emphasizes the increased risk of hearing loss at the frequencies of best hearing (Finneran, 2015a; Houser et al., 2017). It should be noted, however, that frequency weighting was first proposed by Fletcher and Munson (1933) for variations in the perception of loudness of low level sounds rather than a relation to susceptibility to damage. Over the years, A-weighting (I of 5 weightings for humans developed for different sources) has become a standard for estimating workplace exposures, with trade-off of time vs intensity. The appropriate
use of frequency weighting is still debated for impulse sound (Finneran, 2015a, Houser et al., 2017) as well as the applicability to non-humans exposed to a variety of sound sources, although it is lately being applied in marine mammals (Southall et al., 2007; 2019). The frequency at which maximum TTS is observed can vary and is found to be similar to the exposure frequency initially, but shifts to 0.5 octave above the exposing stimulus with increasing SPL (Robles and Ruggero, 2011; Finneran, 2015a, Kastelein et al., 2014b).

2.4.2 Modified power law growth and recovery during intermittent exposures

Equal energy models do not predict the difference in TTS growth in humans due to continuous to intermittent noise exposures, which is likely related to the fact that it fails to account for potential recovery during exposures. A promising approach for predicting the effect of intermittent exposures on TTS growth is the so-called modified power law (MPL) model. This is an “TTS summation” method. In this model, the TTS from individual exposures is raised to some power. It also accounts for recovery between exposures. The modified-power law (MPL) model has been proposed for predicting TTS growth over multiple exposures (induced either by sound, or by medicine) and has been shown to provide good agreement to experimental data for human and terrestrial mammals after exposure to intermittent sounds (Ward, 1973; Humes and Jesteadt, 1991; Macrae 1993, 1994). Finneran et al. (2010) showed that the model can be used to fit TTS growth in dolphins exposed to intermittent tonal noise (Figure 2.4 and 2.5).

Figure 2.4 Example of application of modified power model showing the additive effect of TTS in the presence of existing hearing threshold shift for humans exposed sound in a workplace environment (from Humes and Jesteadt, 1991). The y-axis indicates the amount of TTS induced by a second stimulus vs existing TTS (x-axis). The black lines indicate the MPL model fit to the data for a P=0.15.
Figure 2.5 Example of application of modified power model showing the additivity of TTS in presence of existing hearing threshold shift in dolphins exposed to intermittent tonal sound (from Finneran et al., 2010).

The modified power law model proposed by Humes et al. (1998) is of the form

$$\left(10 \frac{T_N}{10\text{dB}}\right)^p = \left(10 \frac{T_1}{10\text{dB}}\right)^p + \left(10 \frac{(T_{N-1} - T_r)}{10\text{dB}}\right)^p - 1,$$  \hspace{1cm} (Eq. 2.1)

with $T_N$, the amount of TTS induced after $N$ exposures, $T_{N-1}$, the TTS caused by the $(N-1)$-th exposure, and $T_1$ the amount of TTS induced by a single exposure. The recovery term $T_r$ represent the amount of recovery of TTS that occurred since the $(N-1)$-th exposure, and $P$ is a compression term. For humans the factor $P$ was found to lie in the range of 0.1 – 0.3 (Humes and Jesteadt, 1991), although Finneran found a best fit for 2.4 in dolphins (Finneran et al., 2010; see Figure 2.5). In Section 4.1 we explore the possibility of applying the MPL model for predicting growth of TTS in a harbor porpoise due to intermittent exposures, with relatively short intervals (10 – 20 s), such as those used in airgun or sonar operations.

2.4.3 Kurtosis and impulse sound

Recent literature in human and terrestrial mammal noise exposure studies suggests that apart from equal energy rule, empirical corrections can be applied that account for the high pressure peaks during continuous exposures, often expressed in terms of the kurtosis of the signal, which may be more appropriate for impulse sound (Lei et al., 1994; Hamernik and Qiu, 2001; Henderson and Hamernik, 2012; Goley et al., 2011; Zhao et al., 2010, 2016). Kurtosis of an exposure sequence can be interpreted as an effective time in which the exposure occurs (Zhao et al., 2010). A kurtosis-based correction of the equal energy rule better predicted the loss of outer hair cells as a consequence of noise exposure in chinchillas and humans (Goley et al., 2011), and the probability of PTS in human factory workers (Zhao et al., 2010).

Goley et al. (2011) suggested a correction factor to the equal energy rules for predicting risk of NIHL in chinchillas as:
with $\text{CNE}' = \text{CNE} + \lambda \log_{10}\left(\frac{\beta}{\beta_G}\right)$ dB \hspace{1cm} (Eq. 2.2)

Zhao et al. (2010) propose a somewhat different relation,

$$\text{CNE}' = \text{CNE} + K \cdot \log_{10}\left(\frac{T}{1s}\right) \cdot \log_{10}(2) \text{ dB,}$$

with $K = \ln(\beta + 1.9)$, which can be rewritten as

$$\text{CNE}' = \text{CNE} + \lambda' \log_{10}\left(\frac{\beta}{\beta_G}\right) \cdot \log_{10}(T/1s) \text{ dB,} \hspace{1cm} (Eq. 2.3)$$

with $\lambda' = 3.3219$. This is very similar to the Goley et al. (2011) fit, but has an additional $\log_{10}(T)$ factor included.

We will briefly explore whether such kurtosis corrections can explain differences in TTS growth under different exposure (sonar vs impulsive) conditions observed in harbor porpoises in Section 4.2.

2.4.4 Mechanical models for modelling auditory injury

2.4.4.1 The AHAAH model

None of the TTS growth models mentioned in the previous paragraphs provides an explicit link to the underlying mechanisms that cause TTS. The models instead correlate exposure conditions to measured amounts of observed TTS.

Models exist that try to relate the acoustic stimulation to the mechanics of the middle and inner ear in humans and cats. A successful example is the US Army Auditory Hazard Assessment Algorithm for Humans (AHAAH) model (Price, 2007a,b; Price and Kalb, 2018), which was developed specifically to estimate permanent hearing loss (PTS) from shot noise and blasts in humans (with derivative modelling in cats). This model is currently used in practice by various Defence organizations to establish the safe number of allowed training shots and assess the effectiveness of protective gear.

The AHAAH model assumes that the hazard from loud impulse sounds is related to the number of upward deflexions of the basilar membrane (which is assumed to put the stereocilia tip links of the hair cells in tension, which Price and Kalb (2018) speculated to be the most likely mechanical failure mode). The authors argue that the damage should grow linearly for mechanical overstimulation typical for these high amplitude exposures, in contrast to logarithmic growth of TTS that is commonly observed in metabolic overstimulation (Price, 2007a). Frequency-dependent displacement of the basilar membrane is computed using an electric analog that models the outer ear, middle ear, and inner ear (Figure 2.6). The model also
includes two non-linear elements: middle ear muscle response (stapedial reflex) and peak-clipping due to the limited dynamic range in displacement allowed in the middle ear (displacement of the annular ligament of the stapes).

Figure 2.6 Illustration of the AHAAH model and electric analogue of the human ear, used to estimate the impact of gun shots and blasts on humans (image taken from Price and Kalb, 2018).

The AHAAH model expresses the hazard in terms of Auditory Risk Units (ARU), which are defined as the sum of the maximum amplitude squared of the upward displacement of the basilar membrane for each cycle

\[ ARU = \sum D^2, \]

with \( D \) = peak upward displacement (in µm) of the basilar membrane, which are summed over all cycles (interval between zero crossings. These ARUs are computed in the model at 23 locations (with corresponding frequencies) along the basilar membrane. The ARU were experimentally related to probability of hair cell damage for cat and human data (Price, 2003; Price, 2007b) and have shown to better predict risk of the compound threshold shift (CTS) and resulting PTS than the equal energy rule or frequency weighted sound exposure level (see Price and Kalb 2018). The maximum ARUs over all frequencies were found to be related to the amount of CTS observed at 30 minutes after exposure in cats by Equation 2.4.

\[ CTS = 26.6 \ln \left( \frac{ARU}{1 \mu m^2} \right) - 140.1 \quad (Eq. 2.4) \]

For these intense stimuli that result in mechanical damage, the amount of CTS also has a linear relation to the amount of PTS induced and hair cells lost, according to Price (2006), who found that approximately 60% to 80% of the CTS would become PTS (Price and Kalb, 2018).
The AHAHH accurately predicted the frequency encoding locations along the basilar membrane that were affected by hair cell loss in cats (Price, 2003; Price and Kalb, 2018). Frequency of highest membrane response is determined in the model both by the assumed frequency attenuation in the outer and middle-ear, as well as an inactive cochlear inhibitory response (appropriate for high amplitude stimulation). The success of the AHAHH model in predicting the frequencies with maximum response provides a good motivation for using some form of frequency weighting for impulse sound. The model assumes that active processes in the cochlea (i.e. amplification by OHC) do not work for such high amplitude pulses, suggesting a form of frequency weighting which is less steep than the audiogram. It is probable that it is more similar to high equal loudness curves (e.g. Wensveen et al., 2014), making this approach more appropriate. The authors also highlight the need of including the phase information to accurately predict the auditory risk (for example as compared to A-weighted SPL or SEL). As an example, the model could explain that impulse sound with high frequency content (such as gunshots) had a higher auditory damage potential than cannon shots (containing more low frequency energy that does not effectively propagate into the human inner ear), even though both signals have similar peak sound pressures (Price, 1983; Price, 2007).

A practical drawback is that this AHAHH model consists of 69 variables and relies heavily on the knowledge on middle ear, and inner ear properties, which have been well studied in cats and humans, but are currently less well established for many marine mammals (e.g. Miller et al., 2006; Ketten, 2000; Tubelli et al., 2012, 2018; Tubelli and Ketten, 2019). Another limitation of this model with the application to intermittent exposures is that it assumes no recovery between multiple exposures and has not been validated for repeated impulse exposures (Price, 2007a).

### 2.4.4.2 Relationship between the AHAHH model and the MPL model

The AHAHH approach by Price (2007) expresses the growth of CTS due as a function of the sum of the $D^2$ (with $D =$ maximum amplitude of the basilar membrane. We first observe that Price’s relation (Eq. 2.4)

$$CTS = 26.6 \ln \left( \frac{\sum \text{cycles} D^2}{1 \mu m^2} \right) - 140.1,$$

can be rewritten as

$$CTS \approx 10 \log_{10} \left( \frac{\sum \text{cycles} D^2}{1 \mu m^2} \frac{1}{10^{140.1/60}} \right)^6 \text{dB.} \quad \text{Eq. (2.5)}$$

Consider an exposure existing of two discrete sets of exposures consisting of $N$ cycles. As the AHAHH model in its current form does not include recovery, the CTS$_2$ can be expressed as the sum of two exposures

$$CTS_2 = 10 \log_{10} \left( \frac{\left( \sum \text{cycles}_1 D^2 \right) + \left( \sum \text{cycles}_2 D^2 \right)}{1 \mu m^2} \frac{1}{10^{140.1/60}} \right)^6 \text{dB}$$

$$\left(10^{\frac{CTS_2}{6}}\right) = \left( \frac{\sum \text{cycles}_1 D^2}{1 \mu m^2} \frac{1}{10^{140.1/60}} \right) + \left( \frac{\sum \text{cycles}_2 D^2}{1 \mu m^2} \frac{1}{10^{140.1/60}} \right).$$
When we rewrite this equation this relation looks similar to a modified power law relation, with $P = 1/6 \sim 0.16$, which lies well in the range of $P$ values observed in humans (Humes and Jesteadt, 1991).

$$\left( \frac{CTS_2}{10^{10 \, \text{dB}}} \right)^{1/6} = \left( \frac{CTS_1}{10^{10 \, \text{dB}}} \right)^{1/6} + \left( \frac{CTS_1}{10^{10 \, \text{dB}}} \right)^{1/6}$$  (Eq. 2.6)

This suggests that an MPL model can apply to both continuous sound exposures as well as impulsive sound exposures. However, it must be noted that the relationship between continuous exposures and impulse may have different recovery mechanisms, so we would not expect beforehand that the same set of parameters would explain both TTS growth from exposures with continuous and impulse sounds.

2.4.5 Recovery models

The form and degree of recovery depends considerably on the underlying mechanism for TTS growth (summarized in Figure 2.3). The dominant mechanism for the observed TS may change according to the type and degree of exposure (differently for SPL or exposure duration). That is, if the TTS is caused by tip-link breakage, it is the time for tip-link to regenerate. For longer duration exposures, an alternative recovery mechanisms may be the regulation of chemical processes related to excitotoxicity. It is likely that several mechanisms operate simultaneously, with relative importance changing with exposure conditions, making it impractical at this stage to create a bottom-up predictive model for the recovery.

There have been some developments in describing the exponential short term (timescales of few milliseconds) adaptation of HCs (Charasse et al., 2003; Zylani et al., 2009), sometimes also power-law relations used for modelling the long-term (timescales of tens of seconds) adaptation of discharge rates in IHCs (e.g. Zylani et al., 2009). However, so far these recovery models deal with saturation of hair cells and the resulting forward masking (brief elevation of hearing thresholds after sound stimulation; i.e. Harris and Dallos, 1979) and do not incorporate the effects of noise overexposure.

Recovery of TTS is typically measured on timescales of minutes to hours, or even longer. It is generally found that one, or a combination of, logarithmic decay functions can properly describe the recovery of TTS with time (Ward, 1960; Finneran, 2015a). Melnick (1991) notes that when expressed in terms of the ATS, the percentual change follows an exponential function, as $TTS(t) = ATS \cdot \exp(-t/\tau)$. Short-term recoveries of OHC responses on the time scales of hundreds of milliseconds to seconds have been measured indirectly using cochlear microphonics (CM) techniques (Price, 1974), suggesting that a logarithmic recovery with time may be appropriate for these small timescales. However, it has been debated to what extent CM techniques directly measure the OHCs activity (e.g. Perez et al., 2007)
3  TTS growth in harbor porpoises exposed to intermittent and impulsive sound

3.1 TTS experiments with porpoises exposed to impulsive sound in JIP Projects I+II

To investigate the TTS growth in porpoises exposed to airgun sounds, several experiments were carried out with different airgun configurations, as well as animal repositioning at an exposure station and free-swimming exposures. Experiments were conducted at the SEAMARCO Research Institute, the Netherlands. The study animals were kept in a pool complex consisting of an outdoor pool (12 m x 8 m; 2 m deep; Fig. 1) in which the porpoise was exposed to airgun sounds, connected via a channel (4 m x 3 m; 1.4 m deep) to an indoor pool (8 m x 7 m; 2 m deep) in which the hearing tests were conducted. Details of the study area are presented by Kastelein et al. (2017b; in press). The training and testing of the harbor porpoise was conducted under authorization of the Netherlands Ministry of Economic Affairs, Department of Nature Management, with Endangered Species Permit no. FF/75A/2014/025.

This chapter describes the results of data collected in two JIP-funded projects (I) and (II), both aimed at studying Temporary Threshold Shift (TTS) in harbor porpoises exposed to repeated airgun exposures. These experiments have been reported in Kastelein et al. (2017b) and Kastelein et al. (to be submitted to JASA). The following sections briefly describe these exposure conditions, followed by a summary and comparison to other studies.

3.2 Training sessions and determining safe levels of exposure

In total 174 (Project I) and 72 (Project II) sessions (including TTS sessions, control sessions and training sessions) were carried out over a period of approximately 1.5 years. The exposure SELss was increased from conditions that were considered to be safe levels for the porpoise (see Janssen et al., 2015 for the rationale) to levels inducing low levels of TTS by increasing of the firing pressure, total volume, and decreasing the positioning distance of the animal relative to the airgun(s). Sessions were initially repeated testing hearing in a wide frequency range: 0.5, 1, 2, 4, 8, 16, 32, 63, and 125 kHz to avoid missing the TTS onset at a different frequency than the expected one based on the spectrum of the impulsive sound. Due to the unexpected insensitivity of the porpoise to the low frequency content in the airgun sound (Kastelein et al., 2017b), many of these exposure sessions resulted in no TTS over a wide range of tested frequencies. Hence, only a subset of these exposure experiments resulted in small, but significant, amount of TTS, with TTS tested only around the frequencies of maximum TTS (0.5 Hz to 8 kHz).

3.3 TTS growth, recovery and affected hearing frequency range (Project I)

Exposures were carried out with one 3-year-old male harbor porpoise at a listening station, positioned at 1.5 m depth (Figure 3.1). The exposures were carried out with a double airgun set-up, with a mean airgun shot interval: 17.9 ± 4.2 s, maximum SELcum (unweighted: 188 dB re 1 μPa²s; Very High Frequency (VHF); sensu
Southall et al., 2019) weighted: 140 dB re 1 μPa²s; see Table 1). A mean TTS_{1-4} at 4 kHz of 4.4 ±1.9 dB (range 1.9 to 8.4 dB) was observed (Figure 3.2, from Kastelein et al., 2017).

![Diagram of experimental setup]

Figure 3.1 Experimental setup with sound exposures with double airgun set-up during project I.
3.4 TTS increase and dependence on shot interval, duration, and cumulative sound exposure (Project II)

To achieve higher exposure levels than those obtained in Project I, the porpoise was subjected to a 4-gun setup, and the number of shots were increased from 20 to 40 (Figure 3.3; from Kastelein et al., submitted to JASA). It was difficult to achieve exposures with more than 40 shots because the animal was motivated between shots with food; more shots would have decreased the motivation. Therefore, the exposure protocol was adapted to long duration exposures with the animal freely swimming in the outdoor exposure area. In order to achieve similar SELcum, the exposure duration was increased to 90 minutes (1350 shots), with a single airgun (10 in³, with 4 s shot time interval). A single gun was used to avoid stressing the animal. The exposure setup is detailed in Kastelein et al. (submitted to JASA). The acoustic characteristics of the maximum exposure level achieved during the Phase I and II experiments are given in Table 3.1.

The study relied on a porpoise that was trained to participate in a TTS experiment. Project II study was terminated prematurely because of the death of this animal. Unexpectedly, and despite treatment with antibiotics, the animal died of pneumonia (caused by methicillin-resistant *Staphylococcus aureus* bacteria (MSSA)) after showing signs of illness (reduced food intake) for only two days before his death.

Although methicillin resistant infections have been associated with sudden onset of deafness in a few human cases, this has not been reported in other mammals and the deafness coincided with other overt symptoms. Based on a confirmation of normal hearing in the animal during study II (each morning during the study, a pre-exposure hearing test was conducted which showed normal hearing thresholds for the animal), the lack of any prior hearing loss prior to the overt illness, and the results of the post-mortem exam, there is no indication that the animal had any hearing loss related to the infection during the experiments that were performed.
Figure 3.3  Experimental setup with sound exposures with four airgun set-up during Project II.

Figure 3.4  Experimental setup with sound exposures with single airgun and free-swimming animal in the outdoor pool during Project II. The non-study animal was in the indoor pool, on the other side of a bubble screen, which reduced the energy of the high-frequency components of the impulsive sounds (Kastelein et al, in press).
Table 3.1. Characteristics of single shot exposure from the double and four airgun exposures measured at the exposure location.

<table>
<thead>
<tr>
<th>Metric</th>
<th>symbol</th>
<th>Units</th>
<th>Double airgun exposure (mean +/- SD)</th>
<th>Four airgun exposure (mean +/- SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single shot sound exposure level (SELss)</td>
<td>$L_{E,ss}$</td>
<td>dB re 1 µPa$^2$s</td>
<td>178.2 ± 0.6</td>
<td>182.8 ± 0.2</td>
</tr>
<tr>
<td>Weighted single shot sound exposure level</td>
<td>$L_{E,N}$</td>
<td>dB re 1 µPa$^2$s</td>
<td>130.4 ± 1.4</td>
<td>132.5 ± 1.0</td>
</tr>
<tr>
<td>Mean peak sound pressure level</td>
<td>$L_{p,PK}$</td>
<td>dB re 1 µPa</td>
<td>199.1 ± 0.7</td>
<td>202.0 ± 0.8</td>
</tr>
<tr>
<td>Mean peak sound pressure</td>
<td>$p_{PK}$</td>
<td>kPa</td>
<td>9.1 ± 0.7</td>
<td>12.6 ± 1.1</td>
</tr>
<tr>
<td>90 % energy signal duration</td>
<td>$\tau_{90%}$</td>
<td>ms</td>
<td>65.2 ± 3.1</td>
<td>87.2 ± 2.7</td>
</tr>
<tr>
<td>Signal rise-time</td>
<td>$\tau_{rise}$</td>
<td>ms</td>
<td>47.7 ± 3.1</td>
<td>64.7 ± 1.2</td>
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<tr>
<td>Signal rising slope</td>
<td>$r_{max}$</td>
<td>Pa s$^{-1}$</td>
<td>36.9 ± 7.2</td>
<td>34.3 ± 5.5</td>
</tr>
<tr>
<td>Sound pressure kurtosis*</td>
<td>$\beta$</td>
<td>-</td>
<td>32.6 ± 1.7</td>
<td>25.7 ± 0.9</td>
</tr>
</tbody>
</table>

3.4.1 Sound exposure strategy

The harbor porpoise was subjected to airgun sounds presented in six exposure conditions. No TTS was found in the first exposure condition; therefore subsequent conditions were chosen in an attempt to explain this. However, the death of the study animal meant that several exposure conditions were insufficiently replicated to allow firm conclusions.

**Exposure condition 1: facing airguns, shots immediately.** Once the porpoise was accustomed to the maximum SELss produced by the four airguns firing once simultaneously, the number of shots was increased to 10 and the pressure was gradually increased from 4 to 8 bar. In order to keep the inter-shot interval as short as possible (and thus limit the time available for hearing recovery), the airguns were fired as soon as the porpoise was positioned correctly at the exposure station. The porpoise was trained to return to the start buoy and receive a fish reward after each airgun shot. After swallowing the fish he would be sent back to the exposure station immediately.

**Exposure condition 2: facing airguns, shot delay random 1-5 s.** In exposure condition 1, the harbor porpoise may have been able to reduce his exposure to sounds by self-mitigation (achieved by changing the orientation of his head or altering the hearing threshold via processes in the ear or central nervous system), as he knew (either by anticipating a shot within 5 s after being correctly positioned at the exposure station, or from the solenoid click just (4 ms) before the airgun fired) when each shot would be fired (Nachtigall et al., 2018, Finneran, 2018). In an attempt to reduce the animal’s ability to predict when the signal would be produced (and thus prevent him from self-mitigating), the airguns were fired at a randomly selected time between 1 and 5 s after the porpoise was positioned correctly at the exposure station. The number of exposures (at 8 bar, the maximum pressure) per session was increased in steps of 10 shots from 10 (n=1), to 20 (n=3), 30 (n=4) and 40 shots (n=14).
Exposure condition 3: facing airguns, solenoid sound reduced, light reduced. In exposure conditions 1 and 2, the sound of the solenoid valve that triggered the airguns or the LED light in the solenoid connector may have allowed the porpoise to predict when each shot would be fired. Therefore, in exposure condition 3, the sound of the solenoid valve was reduced in one session. A reduction in click level in the one-third octave frequency band SELss of the click by 20-40 dB (mainly above 2 kHz, Figures B3 and B4) was achieved by adding a rubber ring at the interface of the solenoid valve and firing chamber. In addition the light was covered with black plastic tape.

![Figure 3.5 LED light on the airgun connector used for driving the solenoid (left) and when the connector was taped off (right) to avoid providing a cue to the animal.](image)

Exposure condition 4: 1 m from airguns, facing away. In exposure conditions 1-3, the sound pathway to the porpoise’s ears may have been blocked by the dorsal/rostral side of his head (melon and dorsal part of the skull), as the airguns were rostral-dorsal of the porpoises (see Figure 1b); this may have resulted in the lack of TTS. Therefore, in exposure condition 4, the porpoise was trained to position himself facing away from the airguns at the exposure station, which had been turned around 180° but remained at 1 m from the airguns. The sound came from behind the animal (caudal side), and his tail fluke was very close (i.e. below) to the first airgun of the array. Two sessions were conducted.
Figure 3.6 Recorded waveforms at the exposure station for 2 gun setup during Project 1 with old solenoids (top). The same with the new solenoids, measured during Project 2 (middle), and 4 gun setup used during the TTS experiments in Project 2 (bottom). The measured SEL and peak sound pressure level were from top to bottom SEL = \{173 \text{ dB re } 1\mu\text{Pa}^2\text{s}; 178 \text{ dB re } 1\mu\text{Pa}^2\text{s}; 183 \text{ dB re } 1\mu\text{Pa}^2\text{s}\}, and \(L_{p,\text{pk}} = \{195 \text{ dB re } 1\mu\text{Pa}^2; 198 \text{ dB re } 1\mu\text{Pa}^2; 202 \text{ dB re } 1\mu\text{Pa}^2\}.

Figure 3.7 Zoom-in on pre-airgun signal recorded waveforms at the exposure station for 2 gun setup during project 1 with old solenoids (top). The same with the new solenoids, measured during project 2 (middle), and 4 gun setup used during the TTS experiments in project 2 (bottom). The measured SEL and peak sound pressure level within the 3.9 ms period (indicated by the dashed lines) were from top to bottom, SEL = \{112 \text{ dB re } 1\mu\text{Pa}^2\text{s}; 121 \text{ dB re } 1\mu\text{Pa}^2\text{s}; 130 \text{ dB re } 1\mu\text{Pa}^2\text{s}\}, and \(L_{p,\text{pk}} = \{150 \text{ dB re } 1\mu\text{Pa}^2; 159 \text{ dB re } 1\mu\text{Pa}^2; 168 \text{ dB re } 1\mu\text{Pa}^2\}.

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Exposure condition 5: 1.5 m from airguns, facing away. In exposure condition 4, the porpoise was almost touching the nearest airgun (no. 3) with his tail. Therefore, in exposure condition 5, the exposure station was moved so that it was 1.5 m away from the airgun array, so the porpoise’s head was 1.5 m away from airgun 3 in the horizontal plane, still facing away from the array. Three sessions were conducted.

Exposure condition 6: single airgun, free swimming. In exposure conditions 1-5, the porpoise had to return to the start buoy after each shot; after ~40 shots, his motivation declined and it was difficult to continue. Therefore, in exposure condition 6, he was allowed to swim freely in the pool while a single (to reduce unexceptional stress, indicated by very high swimming speeds, only one airgun was used) airgun (no. 1) fired every 4 s (this was the minimum inter-shot interval, as it took about 3.5 s for the airgun to fill with air) for 30 (one at 3, 4 and 6 bar), 60 (once at 3 and 4 bar) and 90 min (twice at 4 bar).

3.4.2 Hearing test signals

The harbor porpoise was asked to detect the hearing test signals before and after exposure to the airgun sounds. Narrowband up-sweeps (linear frequency-modulated tones) were used as hearing test signals. During the psychophysical hearing tests, the same equipment and methods were used as described by Kastelein et al. (2017b). Hearing was tested at the following frequencies: 2, 4 and 8 kHz, as TTS had only occurred at 4 kHz TTS in the previous airgun TTS study with the same study animal (Kastelein et al., 2017b). Daily, before each hearing session began, the SPL of the hearing test signals was checked.

3.4.3 Experimental procedures & data analysis

Daily, one airgun sound exposure session was conducted, consisting of: (1) pre-exposure hearing tests in the indoor pool around 08.30 h, (2) fatiguing airgun sound exposure in the outdoor pool, and (3) post-exposure hearing tests in the indoor pool. For details of the methodology, see Kastelein et al. (2017b).

Video recordings were used to observe the animal’s head position in exposure conditions 1-5, and to track his location in exposure condition 6. To compute the cumulative sound exposure level (SELcum) while the animal was moving, a researcher marked the study animal’s presence in each square in a 1 m x 1 m grid. The SELcum was then calculated by computing the average sound exposure level \( L_{E,ss} \) over three depths (0.5, 1, and 1.5 m), for the pool locations in which the animal swam during the exposure, and integrating this average over the total number of shots \( n_{shots} \), i.e. 
\[ L_{E,cum} = L_{E,ss} + 10 \log_{10} (n_{shots}) \text{ dB} \]

3.4.4 Results

The mean (± SD; \( n = \) number of reversals used to calculate the mean) pre-exposure hearing thresholds of the porpoise were: 74 ± 1 dB re 1 µPa at 2 kHz (\( n = 4 \)), 61 ± 2 dB re 1 µPa at 4 kHz (\( n = 34 \)) and 59 ± 1 dB re 1 µPa at 8 kHz (\( n = 6 \)).

The harbor porpoise’s overall mean pre-stimulus response rates (calculated for all hearing test frequencies and for all six exposure conditions, as the number of responses before a test signal or whistle in a session, divided by the total number of trials in that session x 100) were typical for him: during pre-control threshold measurement sessions: 1%; during post-control exposure measurement sessions:
2%; during pre-airgun exposure measurement sessions: 5%; and during post-airgun exposure measurement sessions: 4%.

The control sessions (in which, instead of airgun shots, a whistle was blown) showed that TTS did not occur after exposure to low ambient noise; the mean TTS$_{1-4}$ for both 2 and 4 kHz was close to zero (1.5 ± 0.4 dB, n=2 and -0.9 ± 2.3 dB, n=5, respectively).

### 3.4.4.1 Exposure condition 1: facing airguns, shots immediately

After exposure to 10 successive immediate quadruple airgun shots, while he was at the exposure station facing the airgun array (SEL$_{cum}$: 193 dB re 1 µPa$^2$s; weighted SEL$_{cum}$: 143 dB re 1 µPa$^2$s; mean (±SD) shot interval: 16 ± 8 s), the harbor porpoise experienced no TTS$_{1-4}$ at 4 kHz (1.4 ± 1.9 dB, n=7). Hearing was not measured at 2 and 8 kHz.

### 3.4.4.2 Exposure condition 2: facing airguns, shot delay 1-5 s

After exposure to 40 successive quadruple airgun shots, delayed by 1-5 s, while he was at the exposure station facing the airgun array (SEL$_{cum}$: 199 dB re 1 µPa$^2$s; weighted SEL$_{cum}$: 149 dB re 1 µPa$^2$s; mean (±SD) shot intervals 15 s ± 1 s), the harbor porpoise experienced no TTS$_{1-4}$ at 4 kHz (-0.1 ± 2.3 dB, n=10), at 2 kHz (-0.7 ± 0.8 dB, n=2), or at 8 kHz (-0.1 ± 0.5 dB, n=2).

### 3.4.4.3 Exposure condition 3: facing airguns, solenoid sound reduced, light reduced

After exposure to 40 successive quadruple airgun shots, delayed by 1-5 s and with reduced cues from the solenoid, while he was at the exposure station facing the airgun array (SEL$_{cum}$: 199 dB re 1 µPa$^2$s; weighted SEL$_{cum}$: 149 dB re 1 µPa$^2$s; mean shot interval: 13 s), the porpoise experienced no TTS$_{1-4}$ (1.0 dB, n=1) at 4 kHz.

### 3.4.4.4 Exposure condition 4: 1 m from airguns, facing away

After exposure to 40 successive airgun shots, delayed by 1-5 s and with reduced cues from the solenoid, shots while facing away from the airguns (SEL$_{cum}$: 191 dB re 1 µPa$^2$s; weighted SEL$_{cum}$: 149 dB re 1 µPa$^2$s; mean shot interval: 17 ± 3 s), the porpoise experienced minimal TTS$_{1-4}$ at 4 kHz (3.9 and 2.6 dB); in both sessions, hearing recovered within 8 min.

### 3.4.4.5 Exposure condition 5: 1.5 m from airguns, facing away

After exposure to 40 successive airgun shots while facing away from the airguns, with reduced cues from the solenoid SEL$_{cum}$: 187 dB re 1 µPa$^2$s; weighted SEL$_{cum}$:146.5 dB re1 µPa$^2$s; mean shot interval: 18 ± 6 s), the porpoise experienced no TTS$_{1-4}$ (-0.5 - 1.5 dB, n=3) at 4 kHz.

### 3.4.4.6 Exposure condition 6: single airgun, free swimming

After exposure for up to 90 min to single airgun shots (at up to 6 bar) while swimming freely in the pool (maximum SEL$_{cum}$: 184 dB re 1 µPa$^2$s; weighted SEL$_{cum}$: 144 dB re 1 µPa$^2$s; mean shot interval: 4 ± 0 s), the porpoise experienced no TTS$_{1-4}$ at 4 kHz or at 8 kHz (1.1 dB, n=1, and 0.5 dB, n=1, respectively).

### 3.4.4.7 Hearing recovery

Recovery from the small TTSs that occurred in the present study occurred in all cases within 12 min (Figure 3.2). Other harbor porpoises suffering TTS recovered
within 60 min after sound exposure stopped (in most cases probably earlier; Kastelein et al., 2012, Kastelein et al., 2013a, Kastelein et al., 2014a, Kastelein et al., 2014b, Kastelein et al., 2015a, Kastelein et al., 2015b). Thus, similar TTSs, caused by various fatiguing sound types (noise bands, tones, sweeps, and impulsive sounds) with various exposure levels and exposure times, required a similar or longer recovery time than seen in the present study. This is consistent with what is found in general for marine mammals (Finneran, 2015a).

3.4.5 Variability of TTS growth within individuals

Exposure to up to 40 shots from an array of four miniature airguns, as well as prolonged exposure to shots from a single airgun, did not cause significant TTS in the harbor porpoise, though the sound exposure levels (both unweighted and frequency-weighted) exceeded the levels for which TTS was observed in the same animal in an earlier study with airguns (Kastelein et al., 2017b). Several possible explanations for the differences in TTS observed in the two studies are considered in detail below.

3.4.5.1 Change in the baseline hearing threshold

The baseline audiogram of harbor porpoise M06 at the time of the present study was compared with that at the time of the study by Kastelein et al. (2017a), in order to check for permanent hearing threshold shift (PTS) that may have been caused by the earlier airgun exposures (Kastelein et al., 2017b). The mean hearing threshold at 4 kHz at the time of the Kastelein et al. (2017a) study (Project I) was 65 ± 1.2 dB re 1µPa². The mean hearing threshold at 4 kHz at the time of the present study was 63 ± 1.7 dB re 1µPa². Therefore, the baseline hearing threshold of the study animal was unchanged, and there was no evidence of PTS at any frequency that could account for the response difference between the two studies.

3.4.5.2 Different signal characteristics

The characteristics of the signals used in sound exposure differed somewhat between the present study and that of Kastelein et al. (2017b). The SELcum, weighted sound exposure, and peak sound pressure were all slightly higher in the quadruple airgun set-up. Signal rise time, duration, and rise rates were similar but slightly higher for the quadruple airgun set-up. If differences in these exposure metrics would lead to different growth of TTS, one would expect a trend with increasing TTS with higher exposure and rise times, rather than less TTS. It was therefore deemed unlikely that these differences in signal characteristics caused the difference in TTS we observed between the two studies.

3.4.5.3 Self-mitigation

The study animal may have been able to regulate or diminish exposure, reducing response sensitivity to the airgun sounds, by a process of self-mitigation. We searched for signs of de-sensitization: changes in the level of TTS observed over time within each of the two studies. The airgun study by Kastelein et al. (2017b), in which the study animal showed ~ 4 dB TTS 1-4, took place between April 2016 and January 2017; the present airgun study between April and December 2017. In both studies, no relationship could be found between the date and the level of TTS 1-4. No such relationship was observed in a parallel TTS study (with continuous noise as fatiguing sound) with the same animal (Kastelein et al., 2017c). This suggests that, if self-mitigation took place in the present study, it started at the beginning of the
second airgun study and did not increase or gradually acquire sensitivity control over time during one or both studies.

Self-mitigation can take place in two ways: the animal can change the orientation of its head so that sound levels reaching the ears are reduced, or it can suppress its hearing sensitivity, decreasing its ability to respond to a sound by one or more neurophysiological auditory response control mechanisms in the middle ear, inner ear, and/or central nervous system (see Section 2.3.1).

**Altering head orientation.**

A behavioral change in head orientation relative to the sound source may affect the level perceived by a porpoise, since its hearing is directional (Kastelein et al., 2005). Such behavior has been observed in bottlenose dolphins (*Tursiops truncatus*) in response to airgun sounds (Finneran et al., 2015b). However, specific changes in head orientation relative to the sound source, like those observed by Finneran et al. (2015b) in bottlenose dolphins, were not seen in the study animal. In the quadruple airgun set-up of the present study, the signals came from a wider range of angles than the sounds described by Finneran et al. (2015b; their Fig. 3.1), and the directionality of porpoise hearing is low (Directivity Index < 3 dB) at the main frequencies (< 10 kHz) of the airgun sounds (Kastelein et al., 2005). As the horizontal directivity of hearing is low for these frequencies, and no changes in head orientation were observed in the porpoise via both the top-view and underwater cameras, self-mitigation is unlikely to have been achieved by altering head orientation.

To test for effects of exposure direction, the study animal was positioned facing away from the airgun in exposure conditions 4 and 5; minimal TTS was observed in exposure condition 4 (head 1 m away from airgun 3), and no TTS occurred in exposure condition 5 (1.5 m away from airgun no 3), when the animal was 0.5 m further away from the airguns, and thus received a slightly lower SELcum.

**Suppression of hearing sensitivity**

The study animal may have learned to adapt its hearing after the study by Kastelein *et al.* (2017b), or cues may have been more detectable, so that the sound exposures may have been more predictable in the present study compared to in the previous airgun study (Kastelein *et al.*, 2017b).

The experimental set-up in the present study did not allow us to assess whether or not the porpoise was actively suppressing its hearing sensitivity during airgun exposures. Cues that the study animal may have used to predict the timing of the airgun sounds include: the predictable timing of the airgun shots when the animal was positioned at the exposure station; response to the first airgun shots; sound produced by the solenoid just 3.9 ms prior the air release (this was louder in the present study than in the previous study by Kastelein *et al.*, 2017b), and the LED light on the solenoid. In exposure conditions 2-6, steps were taken to minimize these cues (but possibly not sufficient), yet little or no TTS was observed.

Analysis of the sound produced by the solenoid showed that it was audible to the porpoise. Sensation levels – here defined as the broadband level difference between the click SEL and the hearing threshold expressed as SEL using $L_{E,ht}=L_{P,ht} + 10 \log_{10}(t_{int}/1 \text{ s}) \text{ dB}$, to correct for the porpoise hearing integration time ($t_{int} = 125 \text{ ms}$; Kastelein *et al.*, 2010) – were between 41 dB (single airgun) and
84 dB (quadruple airgun). The sound was unlikely to be masked by the background noise in the SEAMARCO pool (broadband SELs exceeded the measured background levels in the inner pool by 40 - 55 dB; Fig. B1). However, in exposure conditions 3-6, after dampening of the click signal (which reduced the broadband SELs of the click by 20 dB; mainly above 1 kHz), still no TTS was observed. The dampened click was well below the levels used by Kastelein et al. (2017b), but was probably still audible (both sensation levels and signal-to-background noise ratio of approximately 20 dB; Fig. B2).

Any one or a combination of mechanisms for self-suppression of hearing (see Section 2.1.1), simultaneously or sequentially, are feasible for marine mammals. Considering the rapidity of the reported self-mitigation behaviors, and particularly the experimental results reported here for the harbor porpoise, the acoustic reflex is a viable candidate for consideration as potential or partial explanation because it is both a rapid, automatic reflex arc and it results in an essentially simultaneous bilateral suppression of auditory response.

It therefore remains unclear why the apparent reduction in susceptibility to TTS by the animal only started to occur during the second session after the Kastelein et al. (2017b) experiments. We considered it unlikely that the duration of the experiments had something to do with the decreased sensitivity, because hearing conditioning experiments with porpoises indicate that the harbor porpoises can be trained to reduce its sensitivity within a short (1-3 days) period (Nachtigall et al., 2016). It also remains unclear why the animal was not able to reduce its hearing sensitivity during the sonar exposures that were carried out before, concurrently with, and after this study (Kastelein et al., 2017b). The increased level of the solenoid is suspected to be the main cause, as it serves as a warning signal that the animal may have learnt to associate with the upcoming loud airgun signal. Possibly the animal learned to associate this sound and reduced its sensitivity even after the level of the solenoid signal was reduced later on in the experiment. Additional possibilities are that, over time, the animal developed an aversion to one or more of the test sounds and began self-mitigating in the new study or that one of the new sounds in the second study was particularly unpleasant, like the aversion humans have for chalk squeaks on a blackboard.

Conservatively, we can consider that the immediate responses observed may be explained by a middle ear reflex that then is succeeded by a higher level process and potentially longer term learned response. In the absence of further behavioral experiments and species specific modeling of these proposed systems, the exact mechanisms remain uncertain. Further studies are required to determine the cause and mechanism behind the reduced sensitivity of the porpoise to airgun sound.

### 3.5 Summary of TTS growth in harbor porpoises observed in different exposure conditions

The data collected in Projects I and II adds to a relatively small set of studies aimed at investigating the growth of TTS in porpoises exposed to impulsive sound. The data existing prior to this study was for single airgun exposure (Lucke et al., 2009), and pile driving playback (Kastelein et al., 2015b) (summarized in Table 3.2).
Table 3.2. Summary of TTS studies with harbor porpoises exposed to impulsive sound.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Exposure conditions</th>
<th>Maximum TTS measured (method used to test TTS)</th>
<th>Frequencies Tested (kHz)</th>
<th>Number of shots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucke et al. (2009)</td>
<td>Single Airgun</td>
<td>20 dB at 4 kHz (AEP)</td>
<td>4, 8, 125</td>
<td>1 shot</td>
</tr>
<tr>
<td>Kastelein et al. (2015b)</td>
<td>Pile driving playback</td>
<td>3.6 dB at 8 kHz (behavior)</td>
<td>0.5, 1, 2, 4, 8, 16, 32, 63, and 125 kHz</td>
<td>2760 playbacks</td>
</tr>
<tr>
<td>Kastelein et al. (2016)</td>
<td>Pile driving playback</td>
<td>5.4 at 8 kHz (behavior)</td>
<td>0.5, 1, 2, 4, 8, 16, 32, 63, and 125 kHz</td>
<td>8280 playbacks</td>
</tr>
<tr>
<td>Kastelein et al. (2017b) (Project I)</td>
<td>Airgun (1-4 airgun array) (JIP-project 1)</td>
<td>4.5 dB at 4 kHz (behavior)</td>
<td>0.5, 1, 2, 4, 8</td>
<td>1 – 20 shots</td>
</tr>
<tr>
<td>The present study (Project II)</td>
<td>Airgun (4 airgun array)</td>
<td>n.s. (behavior)</td>
<td>0.5, 1, 2, 4, 8</td>
<td>1 - 40 shots</td>
</tr>
<tr>
<td>The present study (Project II)</td>
<td>Single Airgun</td>
<td>n.s. (behavior)</td>
<td>0.5, 1, 2, 4, 8</td>
<td>1 - 1350 shots</td>
</tr>
</tbody>
</table>

In Kastelein et al. (2017b) these different studies were compared. The main conclusion from that study was that frequency weighted SEL provided a reasonable predictor for the frequency range at which TTS onset was observed, as well as the weighted SEL predicting the onset of TTS (Figure 3.8). The weighted SELcum was in support of the NMFS (2016) and Southall et al. (2019) criteria. Peak sound pressure was found to less predictive for TTS onset. The peak sounds pressure \((L_{p, pk} = 199 \text{ dB re } \text{ dB re } 1 \mu \text{Pa}; \text{ Kastelein et al., 2017}; L_{p, pk} = 202 \text{ dB re } \text{ dB re } 1 \mu \text{Pa in Kastelein, in review with JASA})\) was comparable to those observed in Lucke et al. (2009), even though TTS was not observed for single exposures. The differences in growth rate between frequencies could not be assessed, as no sufficient TTS could be induced.
Figure 3.8 TTS as a result of repeated airgun exposures (black) and pile driving playbacks (gray). The top panel indicates the cumulative decade (one-third-octave base 10) band exposure, the middle panel the VHF-frequency weighted SEL (using Southall et al., 2019), and the bottom panel shows the mean observed TTS1-4. Taken from Kastelein et al. (2017b).
4 Modelling TTS growth for porpoises exposed to intermittent sound (Case Studies)

In this section we explore how models for TTS growth based on the literature can be applied to TTS growth for harbor porpoises exposed to intermittent sound exposures, and whether these can explain differences in TTS growth for exposure durations, duty cycles and exposure SPLs (or SELss). In this section, two different approaches are explored that were selected based on our review in Chapter 2: the modified-power-law (MPL) model, and a kurtosis-corrected equal-energy approach.

Due to the lack of data that could be achieved in this study of airgun exposures causing larger amounts of TTS (> 10 dB), we evaluate these models using previously collected data of porpoises exposed to intermittent and continuous low-frequency (1-2 kHz) sonar sound (Kastelein et al., 2014a, see Figure 4.1). The benefit of this set, is that it has similar exposure intervals as seismic surveys, and a wide range of exposure conditions (SPL, duration, and SELcum), and covers a wider range of TTS induced (up to 33 dB). After fitting the model, we compare the model predictions against the TTS growth for airgun exposures (Kastelein et al., 2017b), pile driving playback (Kastelein et al., 2015b), and sonar exposures at higher (6-7 kHz) frequencies (Kastelein et al. (2015a).

![Figure 4.1 Summary of porpoise data TTS growth as function of number of sounds for different duty cycles, and SPLs using 1-2 kHz 1 s hyperbolic-frequency modulated (HFM) down-sweeps (from Kastelein et al., 2014a) used for fitting the TTS growth models in this study. Each symbol indicates the mean observed TTS, measured 1-4 minutes after the end of each exposure.](image-url)
4.1 Modified power law model

In this section we evaluate how the MPL model can be applied to porpoises exposed to intermittent and continuous sound exposures, and whether it explains differences in TTS growth for exposure durations and exposure SPLs. The objective is to fit these growth functions to harbor porpoises data in which animals were exposed to intermittent and continuous sonar sounds. We build on the MPL as presented in Humes et al. (1998), by considering observed generic relationships of TTS growth and recovery rates with exposure conditions (Finneran, 2015a) and then parameterizing these relationships and fitting them to available harbor porpoise data. For this purpose, the original MPL model needed to be extended in order to make it generally applicable to a wide range of exposure conditions (intermittent as well as continuous). This was achieved by introducing a reference timescale for which acoustic exposure metric (SEL in this case) could be related to the amount of TTS growth over this timescale. The model was fitted to a reasonably sizeable set of TTS data from 1-2 kHz sonar exposures (Kastelein et al., 2014a). Further comparison was then done with other datasets collected on TTS growth, including higher frequency sonar exposures (Kastelein et al., 2014a), pile driving playback (Kastelein et al. (2016), and the airgun exposures collected in the Project I study for the JIP (Kastelein et al., 2017b).

4.1.1 Model assumptions

The modified power law model proposed by Humes et al. (1998) is of the form

\[
T_{NN}^{10dB} = \left[ \left( \frac{T_1^{10dB}}{10dB} \right)^p + \left( \frac{(T_{NN-1}-T_r)^{10dB}}{10dB} \right)^p \right] - 1 \quad (\text{Eq. } 4.1)
\]

For Eq. (4.1) to be useable, the threshold shift due to a single exposure \( T_1 \), and the ‘recovery’ of the threshold shift at the time of the next exposure \( T_r \) need to be measured directly (Humes and Jesteadt, 1991; Finneran et al., 2010). However, in the case of harbor porpoises exposed to repeated, intermittent airgun and sonar sounds, TTS was only measurable after multiple exposures (Kastelein et al., 2014a,b, 2015; Kastelein et al., 2017b; to be submitted to JASA), hence \( T_1 \) could not be directly measured. A second challenge was that seismic and sonar exposures typically have short repetition times, in the order of 5 – 20 s. Recovery rates at those time scales are hard to measure directly (see Section 2.4.5). In order to make use of Eq (4.1), therefore \( T_1, T_r \) and \( P \) have to be inverted for from the TTS measured after multiple exposures. To allow such estimation, relationships between exposure conditions and TTS growth and recovery have to be hypothesized.

The MPL describes how the threshold shifts caused by individual exposures add but does not specify what causes the TTS. Exposure durations differ between the stimuli considered here (few hundred milliseconds to seconds for intermittent airguns and sonar) and become somewhat arbitrary defined for continuous noise exposures. It is therefore not obvious what constitutes a 'single exposure' that should be added by the MPL model. In this study we explore whether the MPL model can be generalized to apply to exposure conditions with arbitrary duty cycles by representing continuous sound exposure as consecutive sub-exposures with a 100% duty cycle (i.e. no recovery throughout the exposure). To achieve this, a time scale was chosen over which sub-exposures of the continuous sound exposures are summed with the MPL model.
The challenges of making the MPL model applicable to TTS growth in harbor porpoises can be summarized as follows:

- The compression factor $P$ in Eq. (4.1) needs to be determined. Is there a rationale for the choosing a particular value for $P$?
- What exposure characteristics should be adopted (e.g. SEL, SPL), how is this related to $T_1$ and what timescale to adopt for determining $T_1$?
- Differences in TTS growth rates are reported for different frequencies, and maximum TTS can shift to higher frequencies when exposure SEL increases. Therefore, growth rates are potentially frequency dependent.
- What relationships to adopt for recovery time? The recovery $T_r$ has also not been measured on short scale, but typically on orders of several minutes or much longer. Are the underlying mechanisms for recovery the same on those time scales?
- If different mechanisms are at play in how TTS is generated by impulsive and continuous sounds, it is not obvious whether the TTS growth and recovery functions in a similar manner.
- It is currently not possible to model the effect of mechanisms as reported for human noise exposures, such as cochlear toughening, or reflex mechanisms that suppress sensitivity.

4.1.1.1 TTS growth from individual exposures, and timescale for TTS growth

In the adapted MPL model, the measured exposure is related to its contribution to the TTS growth ($T_1$ in the MPL model) on a chosen unit reference timescale $t_{ref}$. In principle, the exposure can be quantified in different ways, e.g. either SEL within this timescale, but other parameters such as the peak sound pressure level or sound pressure level could be used as well. For instance, the AHAAH model (Chapter 2) relates the CTS growth to the maximum displacement of the basilar membrane, which is likely more related to the frequency weighted sound pressure measured in a smaller frequency band over the relevant timescale of the hearing system.

Several studies showed that the TS typically increases exponentially with SEL (Finneran, 2015a). We therefore chose to correlate the amount of additional TTS induced to the sound exposure level, $L_p,E,1$, measured on a short reference timescale $t_{ref}$ using the following relationship,

$$T_1 = a \log_{10} \left( 1 + 10^{(L_p,E,1-b)/10} \right) \, \text{dB},$$

(Eq. 4.2)

with parameters $(a, b)$ to be fitted from data.

As stated above, a time duration for the individual intermittent exposures in the continuous sound needs to be chosen. An option would be to use a timescale related to the hearing integration time, or shorter timescales such as the dynamic timescales of neurons (~ 1.4 ms, as in Zylani et al., 2009), or per cycle of the BM vibrations as in the AHAAH model (Price, 2007a). Here, we considered as the base unit of exposure the integrated energy within the integration time of the mammal auditory system. For this analysis, we selected an approximate average of the measured integration times for signals below about 10 kHz: $t_{eq} = 200$ ms (Kastelein et al., 2010). $L_p,E,1$ is the sound exposure energy within that time window.
The effect of choice of reference timescale is discussed in Section 4.1.4.

Figure 4.2  Schematic representation of the TTS growth model for intermittent exposures with \( t_{\text{rec}} \) silence during the exposure, based on the MPL model. The model considers a unit reference time \( (t_{\text{ref}}) \), over which the contribution to TTS is computed, which is then added over multiple time units in which are summed using the MPL model (Eq. 4.1).

4.1.1.2 Compression factor \( P \) in the MPL model

According to Humes and Jesteadt (1991) the MPL model “maintains that threshold sound levels, or their corresponding sound intensities, are not themselves linearly additive. Rather, simple addition holds after the threshold has been nonlinearly transformed to a quantity corresponding to the internal effect associated with the threshold-level stimulus.” This “internal effect” referred to by Humes and Jesteadt (1991) can be interpreted as a reduction in sensitivity, or gain, and from an elevation of the baseline above which sound pressure becomes effective in driving the auditory system (Neubauer and Heil, 2004).

There are different mechanisms that lead to a non-linear relationship between input stimuli and hearing threshold shift. A compressive relationship exists underlying the process resulting in firing patterns of neurons (Heil and Neubauer, 2010). Heil et al. (2011) demonstrated that the spike rates in auditory-nerve fibers (ANFs) (leading to perception of the sound) from the IHC on the sound pressure level of acoustic stimuli follows a power-law of \( R \sim P^\beta \). They find that the best fitting value for \( \beta = 3 \) when the auditory system is driven linearly (i.e. low SPL). Such a relationship implies a \( P=1/3 \) relationship in the MPL model. Heil and Neubauer (2010) argue that different relationships reported between the rate and sound pressure (\( \beta = 1-5 \)) may be explained by saturation of neurons which leads to an apparent change in this relationship, which would imply a change in \( P \). Another important factor that causes compression to occur, is the amplification by OHCs (Robles and Ruggero, 2001). The displacement of the basilar membrane, \( D \), is non-linearly related to the exposure SPL, \( L_{p,\text{exp}} \), by \( \log_{10}(D) \sim 1/B L_{p,\text{exp}} \) (Robles and Ruggero, 2001). In different mammals (chinchillas, cats and Guinea pigs), it is found that for low SPL the relationship is typically linear \( (B=20) \), but for higher SPL the basilar membrane displacement scales with SPL are \( B \sim 40-60 \) (Robles and Ruggero, 2001), and
that B also depends on the location along the basilar membrane, where compressive non-linearity is stronger near the base (higher frequencies) and less near the apex (lower frequencies). This implies that the compression phenomena along the BM is approximately \( P = \frac{1}{3} \) (for \( B = 60 \)) to 1 (for \( B = 20 \)). Since both mechanisms (compression by BM dynamics and the non-linear relationship between BM displacement and neuron firing rates) can act at the same time. It can therefore be expected that the compression factor is not necessarily a constant, but may lie in the range of values for P.

In our analysis, we considered a range of possible compression factors in the range of \( P = 0.1 \) to 3 (the observed range for human TTS studies, Humes and Jesteadt, 1991) and dolphins (Finneran et al., 2010) using Bayesian inference to obtain the P which provides the best fit to the porpoise TTS data.

4.1.1.3 TTS recovery between exposures

We attempt to fit the recovery term between pulses assuming a single logarithmic decay with time after exposure, with decay rate \( m \) that is dependent on total amount of TTS caused by previous exposures (as generally observed in marine mammals, Finneran, 2015a):

\[
T_r = m \cdot \log_{10} \left( \frac{t_{\text{rec}}}{t_{\text{ref}}} \right) \text{dB}, \quad \text{for } t_{\text{rec}} \geq t_{\text{ref}} \\
T_r = 0 \text{ dB}, \quad \text{for } t_{\text{rec}} < t_{\text{ref}}
\]  
*(Eq. 4.3)*

with \( m = c_1 \cdot T_N + c_2 \), \( t_{\text{ref}} = 0.2 \) s, and \( t_{\text{rec}} \) the duration of silence between exposures. It is assumed that no recovery occurs for consecutive exposures periods when signal durations exceed the integration time of our unit exposure \( (T_r = 0 \text{ dB}) \). After the final exposure, \( T_r \) is computed for a period of 4 min (up to the point at which TTS was measured) (Figure 4.2). Here the parameters \( (c_1 \text{ and } c_2) \) are obtained from fitting the model to TTS growth data.

4.1.2 Applying the MPL model to 1-2 kHz sonar - fitting the model parameters

Statistical fitting of the model was carried out with using a Gibbs sampling routine (JAGS in R). The best fit for the parameters (Table 4.1, Figure 4.3) resulted in reasonable match of growth functions for the 100% and 10% duty cycles, and for the varying duty cycle (Figure 4.4). The lower growth rates for 10% duty cycle, as well as the similar growth rates for different exposure conditions (combinations of SPL and exposure duration) can be fitted with the MPL model.
Figure 4.3 Posterior-probabilities for the best fit of the different -parameters for the MPL model fitted to (1-2 kHz) LFAS continuous and intermittent sonar TTS growth using Bayesian inference with a Gibbs sampling routine (JAGS).
Table 4.1 Best-fit and uncertainties of the MPL model parameters to the 1-2 kHz sonar TTS growth data using JAGS in R.

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>Mean</th>
<th>SD</th>
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<th>75%</th>
</tr>
</thead>
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<tr>
<td>$P$</td>
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<td>0.03</td>
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<td>1.82e-1</td>
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<td>$a$</td>
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<td>0.056</td>
<td>-2.19</td>
<td>-2.16</td>
</tr>
<tr>
<td>$b$</td>
<td>158.3</td>
<td>0.76</td>
<td>157.8</td>
<td>158.6</td>
</tr>
<tr>
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<td>3.3e-5</td>
<td>6.04e-5</td>
<td>1.00e-4</td>
</tr>
<tr>
<td>$c_2$</td>
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<td>7.17e-5</td>
<td>5.32e-5</td>
<td>1.44e-4</td>
</tr>
</tbody>
</table>

Figure 4.4 Measurements (color) and model predictions (black) of TTS growth as a function of number of pulses (left) and SELcum (right) for various exposure conditions: intermittent (blue/magenta) and continuous sonar exposure (red) using either fixed SPL (168 dB re 1 µPa), or varying SPL. Exposures were 1 s 1-2 kHz HFM down-sweeps, with 5-100% duty cycle) (data from Kastelein et al., 2014a). For these model predictions, the best fit parameters (Table 4.1) were used.

4.1.3 Model predictions for other sound exposures

4.1.3.1 Sonar exposure (6-7 kHz) (Kastelein et al., 2015a)
After the model was matched to the LFAS exposures, we investigated whether the fitted model parameters then predicted the growth for different frequency exposures. The Kastelein et al. (2015a) showed that the 6-7 kHz sonar signals induced a maximum TTS was found at 9.3 kHz. When compared to the TTS data
measured at 9.8 kHz in Kastelein et al. (2015a), the model predicted lower growth than actually observed, especially for the continuous exposure conditions (Figure 4.5).

![Figure 4.5 Predictions of the MPL model (black) compared to TTS growth observed for 6-7 kHz sweeps (Kastelein et al., 2015a) for continuous exposure (top; red) and 10% duty cycle (bottom; blue) using the best-fit parameters from Table 4.1.](image)

### 4.1.3.2 Piling playback exposure (Kastelein et al., 2016)

The exposure model was applied to TTS growth obtained for porpoises exposed to piling playback sounds for periods of 15 to 240 minutes (Kastelein et al., 2016). The slow growth with exposure duration as observed in Kastelein et al. (2016) is predicted using the exposure model from equation 1, although the predicted TTS levels are somewhat lower (Figure 4.6).
Figure 4.6 Predictions of the MPL model for the TTS growth (black) for pile driving exposure compared to observed TTS growth from Kastelein et al. (2016), using the parameters fitted to the 1-2 kHz sonar growth rate data (Table 4.1). An average broadband SELs = 145 dB re 1 μPa²s for each exposure, and a duty cycle of 2760 strikes per hour. Exposure durations ranged between 15 and 240 minutes.

4.1.3.3 Airgun exposure (Project I, Kastelein et al., 2017b)

The MPL model was compared to TTS growth obtained for porpoises exposed to 1 - 10 airgun exposures obtained during Project I (Kastelein et al., 2017b). When comparing the model predictions to the TTS at the frequency at which the highest TTS was observed (4 kHz), the model fitted to the 1-2 kHz sonar TTS growth predicted smaller TTS than those observed for airgun exposures (Figure 4.7).
4.1.3.4 Model predictions for TTS onset SEL level as function of exposure duration

An interesting observation from the MPL model is that it predicted that the SEL onset threshold for TTS (6 dB) was dependent on the exposure duration, both for intermittent as well as continuous sound exposure (Figure 4.8). This appears similar with an observation for dolphins exposed to sonar sound, where the SEL onset threshold appeared to decrease with increasing exposure duration (Mooney et al., 2009). For shorter duration exposures, threshold for TTS onset were predicted to be higher, but for longer duration exposures, the model converged to an equal-energy rule (constant SEL, independent on duration over which the SEL was delivered).
4.1.4 Discussion

The modified power law (MPL) model was considered to be the most promising method to fit TTS growth in different exposure conditions, as it allows for accounting for recovery of TTS between exposures. The modified-power law model in its original form had to be adapted slightly in order to make it applicable to exposures with different durations (short impulse sounds, intermittent sonar sounds and continuous sonar exposures).

The success achieved in this study is that the model can match the growth of a reasonably sizeable dataset of TTS growth in porpoises for sonar sounds. However, the modelling results presented in this section should be considered with some caution and only as a first attempt to combine and compare intermittent and continuous exposures of different types (tonal vs impulse) into one comprehensive model.

It was found that the model could reasonably predict TTS growth over a wide range of exposure conditions (SPL and duration) when fitted to a broad set of 1-2 kHz sonar exposures. However, the model with the parameters from this fit under-predicted the growth of TTS observed for sonar at higher frequencies (6-7 kHz), as well as for impulsive airgun and piling playback exposures. Given what is currently known about the response of mammalian ears to impulse sound and sensitivity at different frequencies, it is not surprising that the model-fit to 1-2 kHz sonar data does not yet yield a good fit to the airgun and pile driving TTS data.
The best-fitting compression factor $P = 0.16$ in the MPL model was found to be consistent with the range of values reported for humans (0.1 to 0.3, Humes and Jesteadt, 1991; Price, 2007), and the range of expected values based on the compressive mechanisms that are believed to underlie the growth mechanism. However, these numbers are smaller than $P = 2.4$ found to best explain the TTS growth for dolphins exposed to intermittent sonar sounds (Finneran et al., 2010). To better understand the compression factor, TTS summation experiments (as in Humes and Jesteadt, 1991), or two-tone masking experiments (as for Humes and Jesteadt, 1989), should be carried out to independently establish the value of $P$ in porpoises.

Some adaptations could be considered in further development of the model. Currently, the SEL measured on the time scale of the hearing integration time (200 ms) was considered to contribute to the TTS growth in the MPL model. The choice of reference time will have a different effect on how much the sound on this timescale adds to the TTS growth (Eq. 4.2). For the continuous 1-2 kHz sonar exposure with constant SPL, the choice of $t_{ref}$ (200 ms vs shorter timescales of 1.4 ms, corresponding to the dynamic timescales of neurons, Zylani et al., 2009) did not affect the TTS growth in the MPL model for the 1-2 kHz sound exposures. However, for impulse sounds such as pile driving and airguns with large variations in amplitude over short timescales (compared to the hearing integration time), as well as frequency of the sound exposure, the choice of a smaller $t_{ref}$ can lead to different TTS growth. Future studies should therefore consider the model sensitivity on adopted timescale for TTS growth in the MPL model.

Other exposure metrics that describe the characteristics of the sound, such as frequency weighted and band-filtered peak sound pressure on a shorter timescale, which more directly relate to the dynamics of the basilar membrane, and may possibly better explain the difference between the TTS growth of different impulsive sounds and higher frequency sonar exposures. For instance, the AHAAH model (Price, 2007a, see Section 2.4.4) assumes an increase in risk that is proportional to the number of cycles, and the amplitude of displacement of the basilar membrane. For high amplitude sound, non-linear effects within the ear can affect the amount of energy reaching the inner ear, hence the time-dependence of the pressure waveform becomes relevant. Considering success of the AHAAH (Price, 2007a) for predicting the TTS and PTS in humans and cats, it is worthwhile exploring the possibility to use biomechanical models (Tubelli and Ketten, 2019) for predicting the dynamics of the basilar membrane, which according to the Price (2007a) model are a good predictor for hearing loss for loud impulse sounds. Such models will be informative for understanding the response of the porpoise hearing to impulse stimuli, which helps to inform and motivate appropriate frequency weighting functions for impulse sound, and can be used to understand the role of middle ear responses on the sensitivity of porpoises to sound exposure.

The current model did not assume TTS growth rates from individual exposures to be frequency dependent. This may explain the slower predicted growth compared to the 6-7 kHz sonar exposure, and should be investigated further. Recent measurements of TTS susceptibility over a wider frequency range (Kastelein et al. 2019a,b) can be considered to further investigate the frequency dependence of TTS growth.
Currently, the best-fit parameters for recovery (c₁ and c₂) suggest a recovery rate on the time scales of the silences considered in this study (10-20 s) that is much smaller than the recovery rate extrapolated from observed longer time scale (minutes to hours) recovery in porpoises (see Kastelein et al., 2015a; Finneran, 2015a). In principle this is not unexpected, as the \( \log_{10}(t / t_{4\text{ min}}) \) term that is typically fitted to long term recovery (Finneran, 2015a) will diverge for small timescales, which seems physically unrealistic. Future approaches should aim to match the recovery relation from short scales to longer time scales in order to be consistent with measured recovery rates for longer time scales at which TTS is actually measured.

More measurements of TTS growth under different exposure conditions are clearly required to obtain more confidence in this model. The benefit of a model framework as the one presented here is that it allows to build hypothesis that can be tested. For instance, the model suggests a deviation from the equal energy rule for relatively short exposures (a few minutes), which can be verified experimentally. The model can also be applied to other species (such as delphinids) for which TTS growth information is available. However, there are likely some inherent limitations to the MPL model. For instance, in other species TTS sometimes shows a delayed growth (i.e. continued growth after the end of the exposure, e.g. Luz & Hodge, 1970; Finneran, 2015a), and processes such as cochlear toughening (Hamernik et al., 2003), middle-ear responses, and afferent feedback are not explicitly included in this model approach (see Chapter 2).

4.2 Kurtosis-corrected sound exposure level

The kurtosis relationships proposed in Goley et al. (2011) and Zhao et al. (2010) were tested using the TTS dataset using sonar exposures, airgun exposures and pile driving playbacks.

For tones as used during the sonar exposures, the kurtosis values are \( \beta \sim 1.5 \) for continuous sound (100% duty cycle) and \( \beta \sim 15 \) for a signal with 10 s repetition time (10% duty cycle). Figure 4.9 shows the correlation between the observed TTS and both SEL and kurtosis-corrected SEL. From this figure, it is clear that the intermittent and continuous exposures are further apart because of the kurtosis correction rather than creating a more similar relationship. However, it is possible that such a relationship for marine mammals may require a different fitting factor \( \lambda \) (here assumed to be \( \sim 3-4 \), as measured in humans).

When comparing different types of exposures (sonar vs airgun/pile driving), it is observed that onset thresholds are in closer agreement after applying the Goley et al. (2011) kurtosis correction to frequency weighted SEL (Figure 4.10). Even better agreement can be achieved when \( \lambda \) is increased from 4 (as observed in humans) to 13 (Figure 4.10).

Our interpretation is that the kurtosis-correction obtained from continuous noise exposures as measured in humans reflects the increased risk with higher amplitude periods within those exposures, rather than accounting for recovery during intermittent exposures. Hence it is not useful for matching the growth curves for intermittent exposures (with silences in between the exposures).
Figure 4.9 Relationship of observed TTS with SEL (top panel), and with kurtosis-corrected SEL using two approaches by Zhao et al. (2010; middle panel) and Goley et al. (2011; bottom panel) for harbor porpoises exposed to 1-2 kHz sonar (Kastelein et al., 2014a). For this comparison we used the values of $\lambda = 3.32$ (middle) and $\lambda = 4$ (bottom), which have been used to match the relationship between SEL and risk of permanent hearing loss in humans and chinchilla's.
Figure 4.10 Relationship between observed TTS with SEL, for sonar (blue; Kastelein et al., 2014a) and impulsive airgun (black solid; Kastelein et al., 2017b, pile driving playback exposures (open black; Kastelein et al., 2015a) observed for harbor porpoises. From top left to bottom right: (a) TTS as a function of (unweighted) SEL; (b) Southall et al.-2019 VHF frequency weighted SEL; (c) Southall et al.-2019 VHF frequency weighted SEL corrected using the Goley et al. (2011) kurtosis correction, with $\lambda=4$, as in Goley et al., 2011); (d) the Goley et al. (2011) with $\lambda=13$, chosen here to bring the measured different TTS growth curves in porpoises in closer agreement.
5 Conclusion

5.1 Measured TTS growth in harbor porpoises exposed to intermittent airgun sound

The TTS experiments carried out with porpoises provided new insight into the susceptibility of porpoises to impulsive sounds such as airguns. The porpoises were found to be much less sensitive to low frequency sound than expected based on the few prior experiments at other frequencies. This is likely attributable to insensitivity of porpoise to the low frequency content of such signals. A comparative analysis between the different exposure experiments showed that frequency weighted SELcum was a good predictor for the amount of TTS, as well as the frequency range in which TTS is generated. This supported the concept of frequency weighted SELcum as a predictor for hearing loss as recently proposed (Finneran, 2016; NMFS, 2018; Southall et al., 2019).

5.2 Reproducibility of TTS

The observation of TTS occurred repeatedly in the first set of experiments (Project I, as reported in Kastelein et al., 2017b), but could not be reproduced in later experiments (Project II). Several possible explanations were considered. The most likely reason for the lack of TTS is some form of self-mitigation by the study animal, which had learned to anticipate the airgun sounds, either by using the acoustic cue produced by the solenoid, or by the context of the exposure. However, given the lack of a direct demonstration and the limited replication in this study, it is clear that this hypothesis could not be proven, and further studies are required. Unfortunately, due to the death of the study animal, more tests could not be carried out in the present study.

To demonstrate whether self-suppression of hearing sensitivity can occur during exposure to airgun sounds, an animal would need to be trained to conduct hearing threshold tests (e.g. using AEP methods) while at an exposure station where it could expect an airgun sound. An elevated audiogram in this situation would demonstrate that the animal is invoking some form of self-mitigation before and during the exposure, and could help elucidate whether self-mitigation is dependent on a cue, or could occur repetitively and responsively over a longer period (especially when the shot times are not predictable).

An alternative strategy would be to carry out airgun exposures while masking the sound produced by the solenoid that is triggering the airgun. The masking signal would need to be transmitted continuously throughout the exposure, and low enough not to cause TTS itself. However, results could be confounded by forward masking as shown in other species (Harris and Dallos, 1979).

The lack of reproducibility of TTS in the study animal highlights the challenge of understanding the development of TTS in marine mammals and even across individuals within a species. Replication of TTS experiments with multiple animals, including animals that are unfamiliar with the stimuli is required in order to obtain robust and representative TTS thresholds for free-ranging animals for use in the
management and regulation to prevent detrimental effects of underwater sound on marine mammals.

5.3 Modelling TTS growth and recovery for intermittent airgun sounds

The summary of mechanisms underlying TTS and PTS serves to show the complexity of the mechanisms for TTS growth, and recovery (Figures 2.1 and Figures 2.2). Although significant advances have been made in the understanding of these processes in humans and terrestrial mammals, the exact functioning is still not well understood. It is likely that several mechanisms are at play with changing importance depending on exposure duration (metabolic exhaustion) and increasing sound pressure (mechanical fatiguing). This makes it not yet feasible to make a direct link to biomechanically motivated growth and recovery functions.

Based on a literature review we identified different possible approaches for incorporating the impulsive and intermittent sound into TTS growth models. We applied two different approaches to TTS growth data collected for harbor porpoises for intermittent and continuous sound exposures: a kurtosis-corrected equal energy model, and a modified power-law growth model.

The modified power law (MPL) model was considered to be the most promising method to fit TTS growth in different exposure conditions, as it can include recovery of TTS between pulses. The model was tentatively adapted to apply to intermitted as well as continuous exposures, by cumulating the TTS over subsequent exposure periods at a time scale corresponding with the auditory integration time. This study was the first attempt to apply the model to TTS growth in harbor porpoises. Some adaptations were necessary to overcome the issue that the TTS induced from single exposures were too low to cause observable amounts of TTS, and recovery on the timescales of the shot interval is not directly measurable with current techniques.

Applying the kurtosis correction that has been proposed for humans and terrestrial mammals to frequency weighted SEL for different intermittent signals led to more consistent predicted TTS onset thresholds, especially when the free parameter $\lambda$ in this model was tuned to the porpoise TTS growth data. The kurtosis-corrected equal-energy method was found to be unsuited for explaining differences between continuous and intermittent exposures, mainly because it does not account for recovery during silences.

Based on generic parametric relationships between TTS and exposure parameters, the model was fitted to measured TTS growth in harbor porpoises. It was found that the model could reasonably predict TTS growth of intermittent and continuous LFAS sonar sounds over a wide range of exposure conditions, but did not yet successfully explain the (limited) dataset of TTS growth for MFAS sonar and intermittent impulse sounds. Although the adapted version of the MPL model considered here should be considered preliminary, the reasonable fit to the observed data for a wide range of exposure conditions looks promising.
5.4 Implications for mitigation and regulation

The results of the present study have implications for the management and mitigation of the effects of airgun sounds on harbor porpoises. This study confirms that harbor porpoise hearing is less likely to be affected by sounds of low frequency, as was already suggested from equal latency curves (Wensveen et al., 2014) and in recent reviews of effects of various sounds on porpoise hearing (Tougaard et al., 2015; NMFS, 2018). It is also in agreement with data on bottlenose dolphins exposed to airgun and watergun impulses (Finneran et al., 2015b). A frequency weighting that accounts for lower sensitivity at low frequencies has already been adopted in new noise criteria by NOAA in the USA (NMFS, 2018), although policy makers and regulators in other countries rely on unweighted SELcum, or M-weighted SELcum as a criterion for TTS and PTS onset (e.g. Southall et al., 2007, 2019; BSH, 2013; Dekeling et al., 2014). Whether regulations that rely on risk thresholds based on unweighted SELcum overestimate the risk of hearing effects depends on the frequency content of sound sources considered. Although the present study was not aimed at identifying the optimal weighting function, the initial results indicate that the frequency-weighting function proposed by NOAA (NMFS, 2018, Southall et al., 2019) provides a reasonably robust measure of low levels of TTS that occur over a range of spectra of impulsive sound sources.

Based on hearing data alone, mitigation to reduce the risk of hearing damage in harbor porpoises should focus on reducing the high-frequency content (> 100 Hz) of broadband impulsive sounds. Measures such as bubble curtains and noise mitigation screens may be more effective for harbor porpoises than previously believed, since they are typically effective at reducing the frequency elements of impulsive sounds above 100 Hz, such as those generated by airguns, detonations, and percussion pile driving (Coste et al., 2014; Bellman et al., 2014; Lee et al., 2016).

The potential for recovery between impulses and effective quiet levels can influence how the risk of especially distant exposures are computed in impact assessments. Such assessments typically consider the cumulative SEL computed over a 24 h period, include all contributions from fainter distant exposures, and ignore silences between exposures. These assessments have the potential to significantly overestimate the risk when these mechanisms are neglected. This study indicate that recovery from the small observed TTSs occurred in all cases within 12 min, which was consistent with fast recovery observed in other studies (Kastelein et al. 2017). Furthermore, with airgun exposures we found somewhat lower TTS onset levels in harbor porpoises (VHF-frequency weighted SEL = 140 dB re 1 µPa²s), than for pile driving study in Kastelein et al. (2015) (144 dB re 1 µPa²s), despite the longer shot intervals in the airgun study compared to the pile driving study. At first sight, this observation seems to contradict the notion that longer shot intervals lead to more recovery, and hence to higher onset levels (i.e. lower risk). However, the pile driving exposure occurred at lower single pulse levels for much longer duration. The complexity of the interaction between level of individual pulses, duration of silence between pulses and total exposure duration in Equations 4.1-4.3 for the TTS growth model, makes clear that such counterintuitive results may occur due to the different influences these parameters have on the TTS growth. Due to the unexpected insensitivity of the animal to low-frequency broadband impulses (i.e. due to frequency-dependent sensitivity, and potential self-suppression of hearing
sensitivity), and resulting small set of significant TTS data, the current study remains inconclusive into the precise interaction of these parameters for impulse sounds. Although the TTS growth model presented in this study appears promising, more observations of TTS are required to fit the model, before being able to inform environmental impact assessments.
6 References


7 Signature

The Hague, January 2020

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A Effective quiet

The concept of effective quiet, i.e. sound levels that are low enough not to cause or contribute to temporary hearing loss, has been known for several decades, and has been investigated in humans (Ward et al., 1976). Due to the lack of data on effective quiet, current models tend to accumulate over all exposures over a long period (e.g. 24 h), which even for low levels may result in an unrealistic high accumulated exposure. Effective quiet has not been measured in marine mammals (Finneran, 2015a).

One of the objectives of this study was to determine the levels for effective quiet in harbor porpoises, in order to inform exposure models that predict the risk of hearing effects due to airgun sounds. Unfortunately, due to the unexpected death of the animal trained for the airgun exposure studies, these studies could not be carried out. This chapter reflects the considerations behind the experimental design, which will be useful for future studies trying to measure effective-quiet with different animals, or species. and hence we summarize how this has been studied in humans, and developed an experimental design how this can be translated to porpoises in captivity.

A.1 Definition of effective quiet

Two different definitions of effective quiet are possible (Ward et al., 1976):

(Definition 1) Effective quiet = “The minimum SPL at which no TTS is induced, regardless of duration”

This definition was cast in terms of a continuous noise exposure with a constant SPL. Here we re-cast that definition in terms of SELss of a single airgun exposure: “For a given duty cycle there exists an “effective quiet SELss”, below which there is no TTS onset, irrespective of SELcum (i.e. irrespective of duration and number of shots)”

(Definition 2) Effective quiet = “the level of sound that just fails to retard recovery from TTS.”

The second definition is a more practical measure, as exposure durations with human (or animal) subjects cannot be carried out for much longer durations than 2 to 8 hours (one working day). There is no obvious reason that this need be the same as that level that just fails to produce TTS, but studies so far suggest that the effective quiet levels are of similar magnitude (Ward et al., 1976).

In the following sections, we briefly consider how effective quiet is measured in humans, and then how this can be measured in porpoises.
A.2 Measuring effective quiet in humans subjects

The experimental set-up in human effective quiet studies consist of humans being exposed to noises of various patterns for 2, 6, or 8 h (Ward et al., 1976; schematically represented in Figure A.1). The treatment protocol followed by Ward et al., 1976 consisted of a loud sound (aimed at inducing TTS) and a low level sound (aimed at establishing effective quiet) which were transmitted in sequence for a certain duration of the exposure (2, 6 or 8 h). These were then followed by a measuring of the TTS induced at several intervals (2, 15, and 120 min after end of the exposure), with the weak exposure transmitting again between the 15 min and 120 min TTS measurements (Figure A.1). TTSs were measured at frequencies at which the highest TTS was expected (based on earlier research). The SPL of the loud noise was 90 dB re 20 µPa with an octave band centred around the test frequency, and the quiet exposures ranged from 30 to 80 dB re 20 µPa. The resulting TTS measured shortly after the exposure (TTS2), and 120 min after the exposure (TTS120) showed that both TTSs only start to increase as the quiet octave band SPL started to exceed 68 dB re 20 µPa or 76 re 20 µPa, depending on the center frequency of the exposure).

An important observation to make here is that the quiet exposure had a 22 dB lower level relative to the exposure pulses, when it started to contribute to the TTS growth, even though the contribution of these quiet exposures had a negligible contribution to the total SELcum. These results show that the quiet exposures play a role in slowing down TTS recovery between intermittent high level exposures, or continued fatiguing of the hearing rather than the damaging potential of these low level exposures on its own.

\[\text{Note that here we refer to measurements of sound in air, and therefore the reference unit of dB re 20 µPa is used here.}\]
Figure A.1 Exposure protocol followed by Ward et al., 1976 to measure effective quiet in humans.
Appendix A

Figure A.2  Effective quiet SPL measurements in humans (from Ward et al., 1976) according to the noise exposure protocol shown in Figure A.1.

A.3 Measuring effective quiet in captive porpoises

Two approaches are possible for testing effective quiet were considered:
- determine the highest SELss at which no significant TTS is found for 24 hours (typically the total accumulation period used for determining SELcum used by regulators (e.g. NMFS, 2018).
- follow the experimental set-up by Ward et al. (1976) for humans.

Although option (1) would be a pragmatic approach, it was not feasible to carry out this experiment in controlled conditions (no outside interference, or distractions to the animals) for 24 hours at the SEAMARCO facility. Limiting the exposure duration to lower, more practical duration, (e.g. 4-6 hours) would not result in conclusive evidence. Instead, the most realistic approach is to follow that of Ward et al. (1976) as it provides a better theoretical context to demonstrate the actual measurement of effective quiet.

In order to test the effective quiet levels of airgun sounds in porpoises, we needed to consider several practical aspects that drive the experimental conditions, and
how to adapt the protocol as proposed in Ward et al. (1976) to porpoises exposed to airguns:
- what test stimuli to use for inducing TTS?
- what frequencies and times to test for TTS?
- what sound pressure levels to use for inducing significant TTS
- what test stimuli and levels to use for testing effective quiet?
- what exposure duration to use?

**Test stimuli**
The TTS experiments carried out so far indicate that TTS growth for a porpoise exposed to impulse sound (airgun and pile driving playback) is strongest at 4-8 kHz (Figure 3.8), suggesting that these frequencies should be used for measuring the TTS for effective quiet.

As the main aim of this study was to assess the effective quiet levels for airgun sounds, the stimuli to generate TTS is not necessarily restricted to an airgun signal. Test stimuli for the TTS generation that were considered: Alternatives that were considered were the airgun (or array of airguns) used in the TTS exposure studies, or playback of octave band noise generated around 4 or 8 kHz.

The current experimental setup made it hard to generate TTS at all (40 shots, 4 guns), and hence was not ideal for the purpose of this study. Octave band noise can be used effectively to generate TTS growth. Kastelein et al. (2012) achieved TTS exceeding 6 dB with porpoises exposed to octave band continuous noise exposures (centered at 4 kHz) for SPL of 124 dB re 1 µPa, 136 dB re 1 µPa, and to 148 dB re µPa, and exposure durations of 120 min, resp. 60 min, and 7.5 min. From it is concluded that the use octave band noise seems the most promising test stimuli.

**Time to measure TTS**
TTS onset after the exposure and short-term recovery can be measured in porpoises typically at (1-4 min) and (8-12 min) (Kastelein et al., 2012; 2017b). However, TTS needs to be significant in order to measure at longer times (2 hours). Measurement at these larger timescales are necessary in order to assess whether the recovery was retarded by the continued low level exposures (Ward et al., 1976). Kastelein et al. (2012) showed that ~ 3 dB TTS was observed 48 min after exposure for the highest and longest exposure (SPL = 148 dB re µPa; 240 min). Therefore, the level needs to be increased to achieve significant TTS at 96 min after the exposure.

Due to the long exposures, a repositioning of the animal at a fixed exposure station is not practically feasible (decreased motivation with feeding), and therefore a free-swimming exposure protocol has to be adopted. This results in variability of the airgun sound field, which can be reduced by restricting the swimming area, or using noise band exposures. The variability was quantified by measuring a scan of the exposure pool for the airgun sound (Figure A.3). For unweighted values, the SEL was a strong function of distance to the source (due to ineffective propagation of the low frequencies). However, the frequency weighted levels were quiet stable in the pool (as these were dominated by the higher frequencies).
Since this study was aimed at establishing the effective quiet levels for airgun sounds, the test stimulus for quiet exposure needed to be impulse sound: airgun, (distant) airgun/pile driving playback, rather than the octave band noise used in the Ward et al. (1976) study. Some practical considerations for a down-scaled airgun were:
- what is the lowest level achievable by the airguns?
- what is lowest pressure for operation?
- how long can the airgun be operated? (how much compressed air can you store?)

**Lowest levels achievable by the airgun**

A scan of the sound field generated by the smallest airgun operated at the lowest pressure was carried out in the outdoor SEAMARCO pool to characterize the minimum levels that could be achieved with the airgun (Figure A.3). The lowest unweighted SELss achievable using the airgun exposures was 143 dB re 1 µPa²s, or Southall et al.-2019 VHF weighted 105 dB re 1 µPa²s, which was approximately 15 dB lower than the levels achieved by the 10 in³ gun, shot at 8 bar (Table A.1).

**Table A.1** Estimated average single shot SELss within pool for free swimming animals. Power average was taken over 1 m and 1.5 m depth. Extrapolation based on measured scan of 5 in³, 5.5 bar, and difference between that set-up, and 10 in³ airgun, measured at 1 m in front of the airgun.

<table>
<thead>
<tr>
<th>Single airgun</th>
<th>SELss dB re 1 µPa²s (unweighted)</th>
<th>SELss dB re 1 µPa²s (Southall et al.-2019 weighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 cu in, 1 m depth</td>
<td>163</td>
<td>120</td>
</tr>
<tr>
<td>8 bar</td>
<td>162</td>
<td>119</td>
</tr>
<tr>
<td>6 bar</td>
<td>159</td>
<td>116</td>
</tr>
<tr>
<td>4 bar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 cu in, 1 m depth</td>
<td>143</td>
<td>105</td>
</tr>
<tr>
<td>2 bar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The estimates for number of shots are made on the following assumptions:
The compressed-air bottle used in this study had a volume: $V_{\text{bottle}} = 9 \text{ liter} = 549 \text{ in}^3$, and was typically filled at a pressure of ~280 bar. Based on practical experience at SEAMARCO the airguns operated normally down to pressures of 80 bar. The effective capacity is therefore $C_{\text{bottle}} = (p_{\text{fill}} - p_{\text{min}}) \cdot V_{\text{bottle}} = (280 \text{ bar} - 80 \text{ bar}) \cdot (549 \text{ in}^3)$. From this the number of shots can be estimated, by $N_{\text{shots}} = C_{\text{bottle}} / (P_{\text{gun}} \cdot V_{\text{gun}})$. From these estimates, the total number of shots with a full bottle are given in Table A.2, for a range of shot intervals. Acoustic measurements indicated that from shot intervals $\geq 4$ s, the airgun acoustic signals were repeatable, and not so well repeatable below this interval, because the airguns needed time to refill. Therefore, 4 s was taken as a minimum shot interval that could be used in practice. Table A.2 indicates that the long exposures considered here (in order of several hours) could easily be carried out with the smaller volumes and lower pressures.
Table A.2  Estimated number of shots from one full tank, for different single gun settings. Total experimental duration for different shot intervals are given.

<table>
<thead>
<tr>
<th>Airgun setting</th>
<th># shots</th>
<th>Exposure Duration (h)</th>
<th>4 s interval</th>
<th>10 s interval</th>
<th>20 s interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 in³, 8 bar</td>
<td>1372</td>
<td></td>
<td>1.5</td>
<td>3.5</td>
<td>7.5</td>
</tr>
<tr>
<td>10 in³, 6 bar</td>
<td>1830</td>
<td></td>
<td>2</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>10 in³, 4 bar</td>
<td>2745</td>
<td></td>
<td>3</td>
<td>7.5</td>
<td>15</td>
</tr>
<tr>
<td>10 in³, 2 bar</td>
<td>5490</td>
<td></td>
<td>6</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>2.5 in³, 2 bar</td>
<td>27450</td>
<td></td>
<td>30</td>
<td>76</td>
<td>152</td>
</tr>
</tbody>
</table>

A.4  Protocol for testing effective quiet in porpoises exposed to airgun sounds

Based on the previous considerations and experimental boundary conditions, the following experimental design for measuring effective quiet in porpoises exposed to airgun sounds was derived:

The experiment basically follows the protocol as described in Ward et al. (1976) with the following adaptations:

- a 4 h exposure scheme was proposed (6 h was impractical with porpoises (due to potential of outside interference), and 2 h would cause too little TTS in order to measure TTS recovery over long timescales of 1-2 hours)
- expose the animal to semi-continuous (45 s on/off) octave band noise centred at 4 kHz. Based on Kastelein et al. (2012) study, we expect TTS1-4 of 10 - 20 dB for an exposure SPL of 148 dB re 1 µPa. Slightly higher levels will be used in order to achieve sufficient TTS at 96 min after the exposure.
- TTS onset after the exposure and short-term recovery can be measured in porpoises typically at (1-4 min) and (8-12 min) and (96 min)
- Use no-airgun as control (baseline TTS growth for intermittent octave band exposure)
- The effective quiet sound is transmitted for 45 s during the off period (Figure A.4) and between the 12 min and 96 min TTS measurements.
- The airgun source is used as the default stimulus signal. The experiment is started at minimum SELss achievable with the airgun. From the pile driving playback experiment TTS onset occurs after 2760 piling events (6 hours), with SELss of 145 dB re 1µPa²s and a Southall et al., 2019 weighted mean SELss of 109.6 dB re 1µPa²s (Kastelein et al., 2015; Kastelein et al., 2017). This indicates that effective quiet levels should be below these single shot values. The minimum achievable levels using the 2.5 cu in, and 2 bar setup leads to 5 - 10 dB lower SELcum values (frequency weighted) than those observed to cause TTS onset (Kastelein et al., 2017b; this study), and approximately 5 dB below those used to induce TTS onset with pile driving playback. Therefore the minimum airgun settings are expected to be safe to use.
  - If these levels are already sufficient to increase TTS, or stall recovery, switch to playback of airgun sound, and adjust level to get comparable frequency weighted levels (probably lower unweighted SELcum, due to inability to produce these loud low-frequency components with a speaker (see Kastelein et al., 2016). Then start decreasing the weighted SELss.
If these levels are below effective quiet, instead keep using the airgun and increase pressure until TTS_{1-4} and TTS_{96} growth increases.

Table A.3 SELcum after 2 h exposure (45 s on/off) and 4 s shot interval for an effective quiet study with a free swimming porpoise exposed to a single airgun with different settings.

<table>
<thead>
<tr>
<th>Single airgun</th>
<th>SELcum dB re 1 µPa²s (unweighted)</th>
<th>SELcum dB re 1 µPa²s (Southall et al.-2019 VHF weighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 cu in, 1 m depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 bar</td>
<td>193</td>
<td>150</td>
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<td>192</td>
<td>149</td>
</tr>
<tr>
<td>4 bar</td>
<td>189</td>
<td>146</td>
</tr>
<tr>
<td>2.5 cu in, 1 m depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 bar</td>
<td>173</td>
<td>135</td>
</tr>
</tbody>
</table>

Figure A.4 Proposed exposure protocol for testing effective quiet in harbor porpoises.
B Results of airgun pre-exposure signal on old (Project I) and new (Project II) solenoids

Figure B.1 Comparison of ddec SEL of solenoid valve clicks with background noise (ambient noise + self-noise) in the SEAMARCO pool (valve click sound and background noise were integrated over the same duration of 3.9 ms).
Figure B.2 Audibility of the pre-airgun signal (3.9 ms within dashed lines of Figure A.2). Comparison of ddec band SEL of the airgun signals for different airgun setups compared to the porpoise behavioral hearing threshold (Kastelein et al, 2010) expressed in terms of the sound exposure level for tonal signals of 3.9 ms duration, showing that pre-airgun signals (i.e., solenoid clicks) were well above the detection threshold.

Figure B.3 Original (left) and modified (right) section of the solenoid used to trigger the airgun. The Modification was carried out by adding a small rubber ring to dampen the two metal sections causing the click. The airguns with modified solenoids were used during the exposure condition 3 in Project II (see Section 3.4.1) to test whether the loudness of the pre-firing click affected the ability of the animal to anticipate the airgun signal by reducing its hearing sensitivity.
Figure B.4 Comparison of ddec band SEL of the solenoids firing in isolation for the reference ('NEW'; solid) and modified (dashed) solenoid, showing that the adapted solenoid signal effectively reduced the high frequency content by 20–40 dB above 2 kHz. The airguns with adapted solenoids were used during the exposure condition 3 in Project II (see Section 3.4.1).