ABSTRACT

Title of Dissertation: IN-AIR AND UNDERWATER HEARING OF DIVING BIRDS

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In-air and underwater auditory thresholds were measured in diving bird species, using behavioral and electrophysiological techniques. In the first set of experiments, the auditory brainstem response (ABR) was used to compare in-air auditory sensitivity across ten species of diving birds. The average audiogram obtained for each species followed the U-shape typical of birds and many other animals. All species tested shared a common region of greatest sensitivity, from 1000 to 3000 Hz. The audiograms differed significantly across species. Thresholds of all duck species tested were more similar to each other than to the two non-duck species tested. The red-throated loon (*Gavia stellata*) and northern gannet (*Morus bassanus*) exhibited the highest thresholds while the lowest thresholds were observed in the duck species, specifically the lesser scaup (*Aythya affinis*) and ruddy duck (*Oxyura jamaicensis*).

In the second set of experiments, both the ABR and psychoacoustics were used to measure in-air auditory sensitivity in one species of diving duck, the lesser scaup. Both approaches yielded audiograms with similar U-shapes and regions of greatest sensitivity

(2000-3000 Hz). However, ABR thresholds were higher than psychoacoustic thresholds at all frequencies. This difference was smallest at the highest frequency tested using both methods (5,700 Hz) and greatest at 1,000 Hz, where the ABR threshold was 26.8 dB higher than the behavioral measure of threshold.

In the third set of experiments, psychoacoustic techniques were used to measure in-air and underwater sensitivity in one species of sea duck, the long-tailed duck (*Clangula hyemalis*). Underwater auditory thresholds were measured for the first time in any diving bird species. Long-tailed duck in-air sensitivity was greatest at 2000 Hz. The ducks responded reliably to sound stimuli underwater, and correctly responded to high intensity stimuli (greater than 117 dB re 1 μPa) with over 80% accuracy at frequencies between 0.5 kHz and 2.86 kHz. The large differences in diving behavior and physiology across bird orders suggest these studies should be extended to other diving bird species in order to understand how well birds hear underwater. These first measurements highlight the need for further investigation into underwater hearing in diving birds, in order to understand underwater hearing mechanisms and begin to develop hypotheses as to how the introduction of man-made noise sources into the aquatic environment may impact these species.

IN-AIR AND UNDERWATER HEARING OF DIVING BIRDS

by

Sara C. Therrien

Dissertation submitted to the Faculty of the Graduate School of the
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Chapter 1: Introduction

Summary

The aim of this dissertation was to explore the in-air and underwater auditory sensitivity of diving birds. Such birds often dive to significant depths, and may have adaptations to use sensory systems, beyond vision, to assist with foraging, navigating, and/or communicating in the water. In this study, I used electrophysiological methods to compare in-air auditory sensitivity across diving bird species, and psychoacoustic methods to compare in-air auditory sensitivity within a single species. This dissertation includes the first systematic measurements of underwater hearing in a bird.

The Biological Challenge

While light, thermal, and other types of energy are significantly attenuated in water, sound travels approximately 4.5 times faster (1,500 m/s) than in air (344 m/s) (Urick, 1996). Because sound propagates so efficiently in water, many aquatic animals have evolved the use of sound for communication, navigation, and foraging while underwater. Just as terrestrial animals have adapted to compensate for the impedance mismatch between air and the fluid-filled inner ear, however, aquatic animals need impedance matching adaptations to detect sound in a medium that very closely matches their tissues. In addition, all aquatic vertebrates have to adapt to greatly increased pressures at depths underwater.

Wholly aquatic animals such as cetaceans (dolphins and whales) and fishes have adaptations for hearing underwater, while amphibious animals (those that live both on land and on water) such as the pinnipeds (seals and sea lions) or anurans (frogs) may have functional hearing both in air and underwater. Many of the mechanisms for hearing underwater are at least partly understood in pinnipeds and anurans, but almost nothing is known of how diving birds, another amphibious group, may be adapted to hearing underwater.

Entirely Aquatic Hearing

Cetaceans

Structure

While the basic mammalian structure of the middle, and inner ear is preserved in the Cetacea, the path of sound to the inner ear has been greatly modified to adapt for the speed of sound and impedance of water (Au & Hastings, 2008). Cetaceans have no pinnae, which could not impact underwater hearing in the same way that they do in air. In air, the function of the pinna is to collect sound, and perform spectral transformations to incoming sounds, which enables the process of vertical localization to take place (Middlebrooks & Green, 1991). In water, pinna function would be degraded, due to the much longer wavelengths of sound. A pinna would also interrupt cetaceans' streamlined shape (Reidenberg, 2007).

Odontocete cetaceans include sperm whales (*Physeter macrocephalus*), orcas (*Orcinus orca*), beaked whales (family Ziphiidae), and dolphins. Their auditory meatus is not air-filled, but instead filled with cellular debris and dense cerumen, while the tympanic membrane is largely atrophied (Ketten, 1997). The mysticete (baleen whales) meatus is also filled with wax and debris, but the canal ends at the tympanic membrane (Fraser & Purves, 1954. The cetacean middle and inner ear are encased in the tympano-periotic complex (TPC), a dense, bony bulla that is connected to the skull by cartilage, connective tissues and fat (McCormick et al., 1970; Ketten, 1997). While it appears that the auditory canal is unlikely to provide a direct pathway to the middle and inner ear, the pathway of sound through the cetacean head is still uncertain (Ketten, 1992). Norris (1967) suggested that sounds enter the odontocete head through the thinned posterior portion of the mandible (the pan bone), and are transmitted via a fat-filled canal to the tympanic bulla. This "acoustic fat" appears to have a very low sound absorption characteristic that is not found anywhere else in the animal's body (Varanasi & Malins, 1971).

Using evoked potentials from the midbrain of four dolphin species, Bullock et al. (1967) found the greatest sensitivity to sound when the sound source was placed on the lower jaw forward of the pan bone region, and a decrease in response to a distant sound source when the lower jaw was acoustically shielded. More recent studies using the odontocete auditory brainstem response and suction cup sound projectors supported Bullock et al.'s conclusion that the region of maximum sensitivity is slightly forward of the pan bone area (Møhl et al., 1999).

Imaging studies in dolphins have identified mandibular fat channels that could serve to capture and intensify incoming sounds in a manner analogous to the external ear canal of terrestrial mammals (Ketten, 1994, 1997). Finite element modeling, based on CT imaging and measurements of the elasticity of tissues in an animal's head, identified a possible second pathway for sounds arriving from directly in front of an animal, entering the head through soft tissues around the tongue, and passing through an opening in the posterior part of the hollow lower jaws, and finally propagating along the mandibular along the mandibular fat bodies to the bony complex (Cranford et al., 2008, 2010).

The role of the middle ear ossicles has been debated in dolphins (Fleischer, 1973; Norris, 1974; Hemila, 1999; Ketten, 2000). Hearing sensitivity of two beluga whales (*Delphinapterus leucas*) was tested behaviorally, and did not vary at depths of 5, 100, 200, and 300 m, suggesting that sound is conducted directly from the head tissue to the inner ears without involving the middle ear, because increasing pressure on the air-filled middle ear would presumably affect its volume and therefore hearing sensitivity (Ridgway, 2001). Once the sound has traveled through the odontocete mandibular fat channel, sound could be coupled to the bulla through bone conduction (McCormick et al., 1970). Other studies have suggested parallel channels; low frequency sounds may be transmitted to the TPC through bone conduction, and higher frequencies through ossicular involvement (Cranford et al., 2010).

Sensitivity

The cetacean species most used for auditory investigations has been the bottlenose dolphin (*Tursiops truncatus*). Johnson (1967) conducted the first systematic measurements of auditory sensitivity in the bottlenose dolphin with a psychoacoustic go/no-go task. Results demonstrated good sensitivity, i.e. within 10 dB of the maximum sensitivity, between 15 and 110 kHz, and a very steep high-frequency roll-off of 495 dB per octave above approximately 110 kHz (Figure 1.1). Since this experiment, auditory sensitivity has been measured in several other species of odontocetes (Figure 1.1). Despite the differences in experimental methods and species, the audiograms of these cetacean species are remarkably similar in shape, levels of maximum sensitivity, and high-frequency roll-off. Generally, on the low-frequency end of the U-shaped audiogram, the roll-off is approximately 10-15 dB per octave. Maximum absolute sensitivity (or the lowest detection level in a quiet environment) for most species ranges between 35 and 55 dB re 1 µPa, and the high-frequency roll-off is generally at least 100 dB per octave.

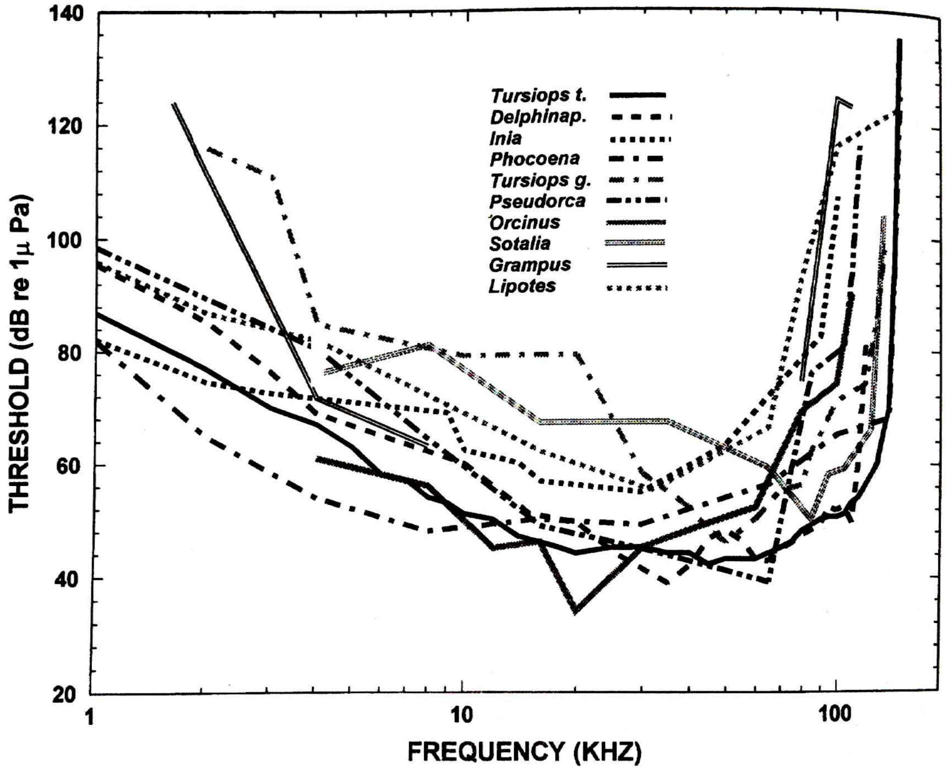

Figure 1.1. Behavioral audiograms from odontocete species, taken from Au & Hastings (2008). Original data from the bottlenose dolphin, *Tursiops truncatus* (Johnson, 1967), the Pacific bottlenose dolphin, *Tursiops gilli* (Ljungblad et al., 1982), the killer whale, *Orcinus orca* (Bain & Dallheim, 1992), Amazon river dolphin, *Inia geoffrensis* (Jacobs & Hall, 1972), beluga, *Delphinapterus leucas* (White et al., 1978), false killer whale, *Pseuodorca crassidens* (Thomas et al., 1988), Risso's dolphin, *Grampus griseus* (Nachtigall et al., 1995), tucuxi, *Sotalia fluviatilis* (Sauerland & Denhardt, 1998), harbor porpoise, *Phocoena phocoena* (Andersen, 1970), Chinese river dolphin, *Lipotes vexillifer* (Wang et al., 1992).

Fishes

Structure

Studies of bony fishes reveal a wide range of adaptations for underwater hearing. Fishes do not have outer or middle ears, and the inner ear has no cochlea or auditory papilla (Popper et al., 1988). The inner ear consists of three semicircular canals and three otolithic end organs – the utricle, saccule, and lagena - that serve both vestibular and auditory functions (Popper et al., 1988). The motion of the otoliths, which are four to five times denser than water, lags with respect to the acoustically transparent body, thereby creating a shearing force that bends the hair cell ciliary bundles on the sensory epithelium, causing stimulation of the auditory nerve (for a recent review, see Popper & Fay, 2011).

Underwater, sound has both particle motion and pressure components (Fay & Popper, 1974, 2000). Most commonly, hearing involves the detection of particle motion, when the sound wave travels through the water and acoustically transparent body (Fay & Popper, 2000). Certain fishes have auxiliary structures that enhance sound pressure transmission to the inner ear. Gas-filled swim bladders, used primarily to regulate buoyancy, vibrate in response to sound and in the Otophysi fishes (a group of fishes with sensitive hearing, including the carps, minnows, catfishes, characins, and knifefishes) transmit this oscillation to the inner ear via Weberian ossicles (von Frish, 1938; Fay, 1988; Fay & Popper, 2000; Ladich, 2000). These accessory structures can lower auditory thresholds and extend the auditory bandwidth by coupling the movements of air spaces to the inner ear.

Anterior swim bladder horns, or extensions of the swim bladder towards the ear, are the simplest accessory structure, and can be found in some holocentrids (squirrelfish) and all clupeids (herrings, shads, sardines, hilsa and menhadens) (Coombs & Popper, 1979; Grande & de Pinna, 2004). Gouramis and mormyrids (African weakly-electric fishes) have air-filled bubbles that touch the inner ear (Yan, 1998; Ladich & Fay, 2013). Popper (1974) showed that the swim bladder of the goldfish had a flat response from 50 to 2000 Hz, corresponding to the auditory bandwidth of that species. This was later confirmed by Finneran and Hastings (2000, 2004), who used a noninvasive ultrasonic vibration measurement system to show that the goldfish's swim bladder resonance frequency and bandwidth correlated with its most sensitive region of hearing.

Sensitivity

The auditory sensitivity of fish is as varied as the structure of the peripheral auditory anatomy across species. Species that possess potential specializations, such as those described above, for sound pressure detection, such as weakly electric fish, otophysines, and gouramis, have lower sound pressure thresholds (55-83 dB re 1 μPa) and broader bandwidth (100 Hz-3 kHz) hearing capability than species without these specializations (Fay, 1988; Ladich & Fay, 2013) (Figure 1.2). Several species (American shad *Alosa sapidissima*, Gulf menhaden *Brevoortia patronus*) have been shown to respond to high intensity ultrasound up to about 200 kHz (Mann et al., 1997, 1998). Species that do not have a swim bladder, such as the elasmobranchs, or teleosts without a connection between the swim bladder and inner ear, generally have best sound pressure thresholds above 90 dB re 1 μPa, and a bandwidth of 100 Hz to 1 kHz (Fay, 1988; Ladich & Fay, 2013). However, auditory thresholds reported in terms of pressure can be misleading for these non-specialized species because all fish detect acoustic particle motion directly with the inner ear as well as with the lateral line, and some species are primarily particle motion sensitive. Therefore, auditory thresholds reported in terms of pressure may not actually represent the detection of acoustic pressure, but rather a combination of acoustic pressure and particle motion, or just particle motion.

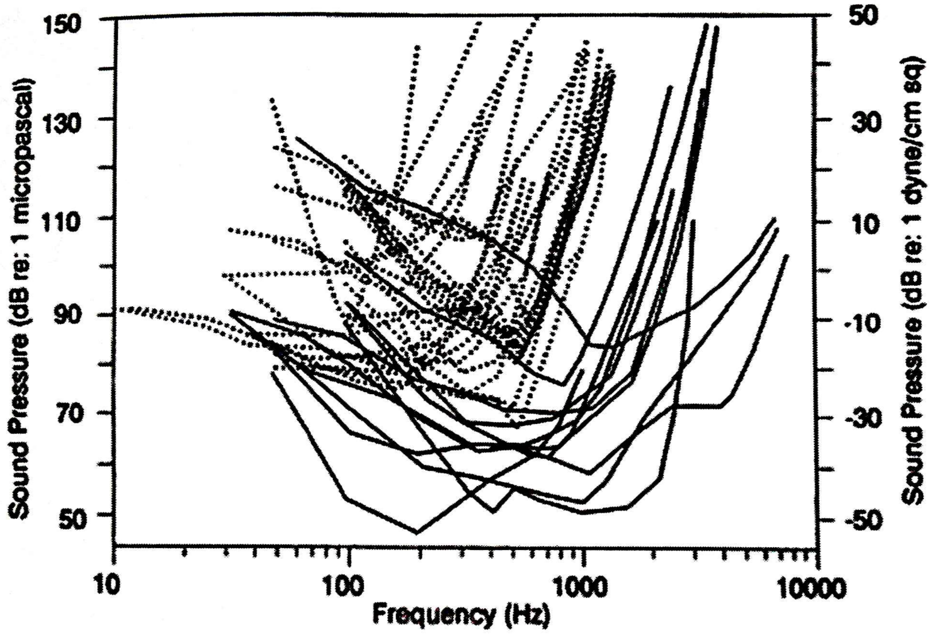

Figure 1.2. Behavioral sound pressure audiograms for various fish species taken from Fay (1988) and Au & Hastings (2008). Dotted lines are considered hearing generalists and solid lines are hearing specialists (from Popper & Fay, 1999).

Amphibious Hearing

Pinnipeds

Structure

There is a 3600-fold difference between the impedance of water and air. In the air, the mammalian middle ear serves to compensate for losses due to this impedance difference between the surrounding air and the fluid-filled inner ear. Underwater, however, this function of the middle ear may not be necessary, and instead, the ear must function to detect sound that can travel through the body.

Phocids (true seals) do not have pinnae, while otariids (sea lions) and odobenids (walrus) have very small pinnae that may function to close the external meatus during diving (Kastalein, 1996). In air, spectral filtering by pinnae plays a significant role in sound localization in the vertical plane, so the lack of a functional pinna may limit some localization abilities (Middlebrooks & Green, 1991). In pinnipeds, the auditory meatus is very narrow and has several bends, and is lined with cerumen and wax-covered hair (Rampreashad et al., 1972; Kastalein et al., 1996). Unlike the dolphins, the external meatus may be functional in pinnipeds, at least in the air (Møhl & Ronald, 1975; Kastalein et al., 1996).

Underwater, an air-filled middle ear would create an impedance mismatch and therefore a loss of sensitivity, therefore it is possible that pinnipeds hear through bone conduction (Repenning, 1972; Ramprashad, 1975; Hemilä et al., 2006), much like a human underwater (Brandt & Hollien, 1969; Hollien & Brandt, 1969; Repenning, 1972). Consistent with this hypothesis, the area of maximum sensitivity on the head appears to be directly below the auditory meatus (Møhl & Ronald, 1975).

Pinnipeds have several adaptations to compensate for increasing pressure while diving, and these may or may not affect hearing abilities. The meatal opening can be closed actively by surrounding muscles while diving, trapping air inside (Welsch & Riedelsheimer, 1997; Stenfors et al., 2000). The bony tympanic bulla and meatus is also covered with cavernous tissue, which is highly vascularized and fills with blood to compensate for increasing pressure on the tympanic membrane as the animal dives (Repenning, 1972; Stenfors et al., 2001). It is possible that when this tissue is flooded with blood on both sides of the tympanic membrane, acoustic conductance could occur through the traditional tympanic route (Møhl, 1968; Repenning, 1972; Moore & Schusterman, 1987; Terhune, 1989). Behavioral hearing measurements of a California sea lion (*Zalophus californianus*) at depths of 10 m and 50 m demonstrated that at 10 m, sensitivity matched surface values, but at 50 m the threshold for high-frequency sounds was significantly lower (Kastak & Schusterman, 2002). This suggests that the sea lion middle ear is functional underwater. If hearing sensitivity did not change with depth, this would suggest that sound is conducted directly to the inner ear without passing through the middle ear (because increasing pressure on the air-filled middle ear would presumably affect its volume and therefore hearing sensitivity) (Ridgway et al., 2001; Kastak & Schusterman, 2002). In the sea lion, high frequency sensitivity in shallow water could be poor because of reflection off the middle ear, but in deeper water, the sensitivity could improve because of the expansion of cavernous tissue on both sides of the tympanic membrane (Kastak & Schusterman, 2002).

Sensitivity

The first measurement of a pinniped audiogram by Møhl (1968) revealed a U-shape with similar characteristics to cetaceans, with a steep high-frequency roll off and a flatter low-frequency roll-off. Maximum sensitivity for this harbor seal was at 32 kHz underwater, and approximately 10 kHz in the air. The harbor seal had better underwater sensitivity and bandwidth than in air. Since this experiment, underwater sensitivity has been measured in the California sea lion, *Zalophus californianus* (Schusterman et al., 1972), harp seal, *Pagophilus groenlandicus* (Terhune & Ronald, 1972), ringed seal, *Pusa hispida* (Terhune & Ronald, 1972), Northern fur seal, *Callorhinus ursinus* (Schusterman & Moore, 1978), Hawaiian monk seal, *Monachus schauinslandi* (Thomas et al., 1988), elephant seal, *Mirgounga angustirostris* (Kastak, 1996), and Steller sea lion, *Eumetopias jubatus* (Kastalein et al., 2005) (Figure 1.3). The in-air sensitivity has been measured for the harbor seal, harp seal, California sea lion, Northern fur seal, and elephant seal (Møhl, 1968; Terhune & Ronald, 1971; Schusterman & Moore, 1980; Kastak, 1996) (Figure 1.4). Auditory bandwidths for all species are wider underwater then in air. Maximum underwater sensitivity is relatively consistent across pinniped species (approximately 60-70 dB re 1 μPa), but in-air hearing varies greatly across species, without any consistent correlation with amount of time spent in the water, diving ability, or sound production (Au & Hastings, 2008).

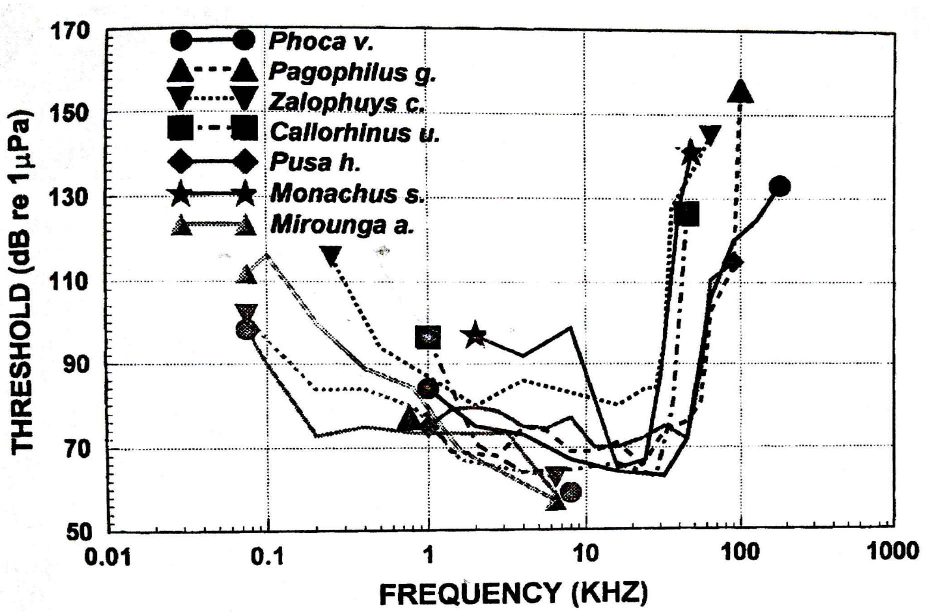

Figure 1.3. Underwater behavioral audiograms from various pinniped species, taken from Au & Hastings, 2008. Original data from the harbor seal, Phoca vitulina (Møhl, 1968; Kastak, 1996), California sea lion, *Zalophus californianus* (Schusterman et al., 1972; Kastak, 1996), harp seal, *Pagophilus groenlandicus* (Terhune & Ronald, 1972), ringed seal, *Pusa hispida* (Terhune & Ronald, 1975), Northern fur seal, *Callorhinus ursinus* (Schusterman & Moore, 1978), Hawaiian monk seal, *Monachus schauinslandi* (Thomas et al., 1988), and elephant seal, *Mirgounga angustirostris* (Kastak, 1996).

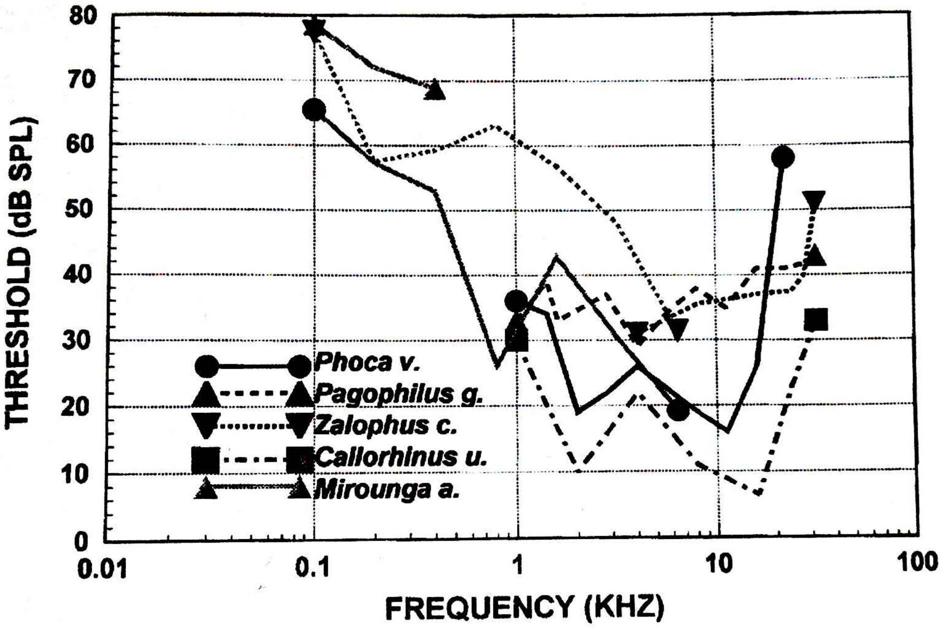

Figure 1.4. In-air behavioral audiograms of various pinniped species, taken from Au & Hastings, 2008. Original data from the harbor seal, (Møhl, 1968; Kastak, 1996), harp seal, (Terhune & Ronald, 1971), California sea lion (Schusterman, 1974; Kastak, 1996), northern fur seal (Schusterman & Moore, 1980), and elephant seal, *Mirgounga angustirostris* (Kastak, 1996).

Frogs and Turtles

Structure

Similar to pinnipeds, some frogs and turtles are amphibious and must contend with hearing both in-air and underwater. They will be considered together here. The anurans do not have pinnae, and many species do not have a tympanic membrane or middle ear cavity (Mason, 2007). In anurans that do have a tympanic membrane, it is typically visible on the head of the frog behind the eye, and the single middle ear bone, the columella, spans the middle ear cavity. Vibrations of the columella are transmitted to the inner ear via the oval window. “Earless” frogs like the European fire-bellied toad, *Bombina bombina* (those without a tympanic membrane, middle ear cavity, and columella), can still have a well-developed inner ear (Jaslow et al., 1988).

Their inner ears may be stimulated via extra-tympanic pathways, including the lungs and mouth cavity (review in Christensen-Dalsgaard, 2005). Some frog species, such as the Ranids, are mainly terrestrial outside of the mating season, whereas others, such as members of the genus *Xenopus*, are fully aquatic and show several specializations for hearing underwater. The *Xenopus* ear is anatomically different from terrestrial frogs (Wever, 1984). Instead of a tympanic membrane, *Xenopus* has a cartilaginous tympanic disk covered by fat and skin and suspended in a membranous frame. Christensen-Dalsgaard et al. (1990) proposed that resonance of the air-filled middle ear in the underwater sound field mediates sensitive responses to sound underwater.

Like the anurans, the testudines (turtles and tortoises) range in degree of aquatic adaptation. Like *Xenopus*, testudines have a cartilaginous tympanic disk, visible behind the eye that vibrates via a hinged connection to the bony capsule wall surrounding it (Wever & Vernon, 1956; Christensen-Dalsgaard et al., 2012). A columella attached to the tympanic disk extends through the large middle ear cavity to the oval window (Willis et al., 2012).

Sensitivity

Christensen-Dalsgaard et al. (1990) found that the *Xenopus* ear was 30 dB more sensitive to sound pressure underwater than in air, suggesting adaptations for hearing underwater, whereas the Ranid ear showed a greater sensitivity in air (Lombard et al., 1981). Christensen-Dalsgaard and Elepfant (1995) hypothesized that air in the lungs vibrates much like a swim bladder in a fish, and that these vibrations are coupled to the middle ear via the larynx.

Using laser vibrometry and the auditory brainstem response, Christensen-Dalsgaard et al. (2012) found that the red-eared slider (*Trachemys scripta elegans)* was most sensitive to sound underwater. The tympanic disc is the sound receiver, and their underwater sensitivity depends on resonance of their large air-filled middle ear. Thresholds in water, when compared in terms of sound intensity, were approximately 20-30 dB lower than in air. Therefore, the sensitivity of the turtle to underwater sound is broadly comparable to the sensitivity of both *Xenopus* (Elliott et al., 2007) and otophysine fish (for review, see Popper & Fay, 2011), allowing for the difference in experimental design and the generally higher thresholds in ABR experiments (see below, chapter 2).

Diving Birds

The class Aves includes 29 orders, and a total of approximately 10,000 species. Eight of these orders include birds that dive to some extent while foraging: Anseriformes (waterfowl), Charadriiformes (gulls and allies), Gaviiformes (loons), Podocipediformes (grebes), Procellariiformes (albatrosses, petrels, and allies), Sphenisciformes (penguins), and Phaethontiformes (tropicbirds). Diving bird species are spread throughout the avian taxa (Figure 1.5). Species used in this dissertation come from three families: the diving ducks in the family Anatidae (ducks, geese and swansa), the red-throated loon (*Gavia stellata*) in the family Gavidae (loons), and the northern gannet (*Morus bassanus*) in the family Sulidae (boobies and gannets).

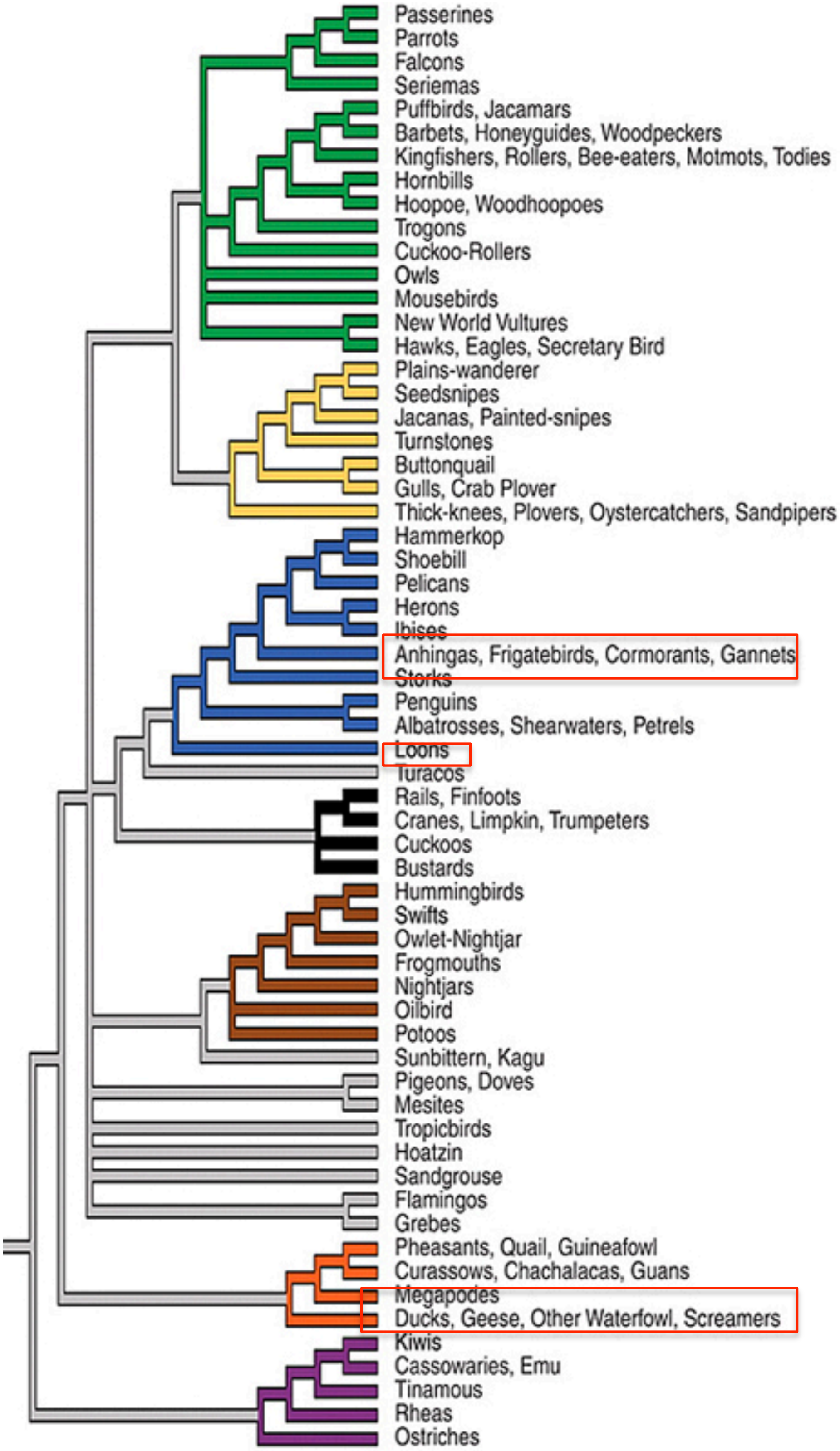

Figure 1.5. Avian phylogeny taken from Hackett, 2008. Boxes surround groups that were used in this dissertation.

Across all diving birds, time spent underwater ranges from minimal plunges in gulls and waders to extensive diving in penguins and puffins. At the shallow end of the diving continuum, the diving ducks can dive to depths of tens of meters for up to a few minutes (Roberston et al., 2002).

At the other end of the continuum are the penguins. The emperor penguin (*Aptenodytes forsteri*) has a recorded maximum dive greater than 500 m and lasting over 23 min (Meir, 2008). In addition, other penguin species demonstrate well-developed diving abilities. King penguins (*Aptenodytes patagonicus*) can dive to depths of over 300 m (Kooyman et al., 1992), gentoo penguins (*Pygoscelis papua*) over 200 m (Bost et al., 1994), and adelie penguins (*Pygoscelis adeliae*) close to 100 m (Chappell et al., 1993). In addition, many penguin species, including adelie, macaroni (*Eudyptes chrysolophus*), and king, dive frequently at night when visibility is low (Croxall et al., 1988; Kooyman et al., 1992).

Species from the family Alcidae (auks, murres, puffins, and guillemots) also dive to great depths. Incidental catches of alcids from fishing nets revealed that common murres (*Uria aalge*), razorbills (*Alca torda*), Atlantic puffins (*Fratercula arctica*), and black guillemots (*Cepphus grille*) can dive to 180, 120, 60, and 50 m, respectively (Piatt & Nettleship, 1985). Underwater cooperative foraging has also been observed in several alcid species including marbled murrelets (*Brachyramphus marmoratus*), least auklets (*Aethia pusilla*), and crested auklets (*Aethia cristatella* (Hunt et al., 1993; Speckman et al., 2003).

Many studies have been conducted to investigate the role of sound in the lives of diving birds while they are out of the water. Most penguin species rely on sound to individually identify their mates and chicks, to attract potential mates, and defend territories. Nesting penguin species, such as the adelie penguin and gentoo penguin, use call pitch to discriminate between individuals in the colony (Jouventin & Aubin, 2002). Little blue penguins (*Eudyptula minor*), a nocturnal, cave-dwelling species, can also distinguish individuals through the use of auditory cues (Nakagawa et al., 2001).

The two non-nesting species, the emperor and king penguins, have evolved more complex methods for individual recognition amongst several thousands of unrelated birds in the tightly packed, noisy colony (Aubin et al., 2000). Emperor penguins cannot identify their mates or chicks visually (Jouventin, 1982). Instead, both species identify their mates and chicks through the use of the "two-voice" system. These species produce two slightly different fundamental frequencies simultaneously which interact with each other and generate a beat (Aubin et al., 2000). This amplitude modulation, along with other temporal and spectral cues, allows each individual to create its own stereotyped vocal signature (Lengagne et al., 2000).

Bird Hearing

Structure

Birds lack pinnae, but have a feather-covered external auditory canal. Feathers covering the canal may be adapted for minimizing air (or water) turbulence during flight or diving, and for waterproofing in diving birds (Rijke, 1970). Like the amphibians and reptiles, birds have a single middle ear ossicle, the columella, connecting the tympanic membrane to the oval window. Diving birds, like other aquatic animals that dive to considerable depths, have ear adaptations for increasing water pressure, such as a cavernous tissue in the meatus and middle ear, and active muscular control of the meatal opening (Sade, 2008). In addition, some alcids have a flap of skin covering the meatus that, when closed due to increased pressure during diving, causes a body of fat to press against the tympanic membrane (personal communication, D. Ketten, 2013).

Sensitivity

The single columella ear allows for efficient transmission of sound (Manley, 1981, 2010), but its efficiency deteriorates at high frequencies (> 4 kHz), and even in birds that are adapted to hearing high frequency sounds, such as the barn owl (Tytonidae), swiftlets (Collocaliini), and oil birds (S*teatornis caripensis)*, the upper frequency limit is less than 15 kHz (Manley, 1981; Manley & Gleich, 1992). Many mammals with high-frequency hearing have an upper limit near 50 to 70 kHz. Thus, the basic structure of a single-ossicle middle ear probably limits the high-frequency limit of bird hearing (Manley, 2010).

Hearing has been measured behaviorally in approximately 50 species of birds (Dooling, 2002). Sensitivity is greatest between about 1 and 5 kHz, with absolute sensitivity often approaching 0-10 dB re 20 µPa between 2-3 kHz (Dooling, 1980, 1982, 1992, 2000, 2002). The passerines (perching birds – includes song birds) on average have more sensitive high-frequency hearing than other birds (Figure 1.6). Nocturnal predators, such as the barn owl and great-horned owl (*Bubo virginianus*) have very low absolute thresholds, as shown by the Strigiformes in Figure 1.6 (Konishi, 1973; Van Dijk, 1973; Dyson, et al., 1998).

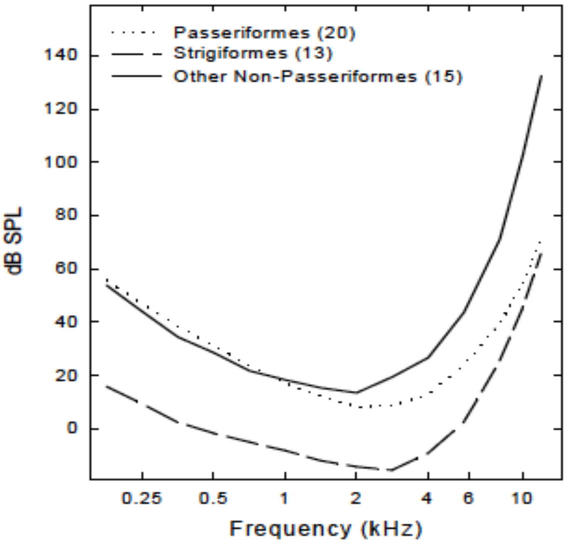

Figure 1.6. Median audiograms, taken from Dooling (2002), for three groups of birds – Passeriformes (perching birds) - 20 species, Strigiformes (owls) – 13 species, and other non-Passeriformes – 15 species. The bird species in this dissertation fall under the "other non-Passeriformes" grouping.

Methods to Test Auditory Sensitivity

Auditory sensitivity can be measured using psychophysical or electrophysiological methods. There are many variations within these two measurement categories, but for this dissertation the focus will be on a comparison of two techniques: a psychoacoustic go/no-go task, and the auditory brainstem response (ABR).

Psychoacoustics

Psychoacoustics has been used to measure auditory sensitivity in many marine mammal species (Hall & Johnson, 1971; Jacobs & Hall, 1972; Schusterman et al., 1972; White et al., 1978; Thomas et al., 1988; Nachtigall et al., 1995; Ridgway & Carder, 1997; Sauerland & Denhardt, 1998; Kastalein et al., 2002; Kastalein et al., 2003; Kastalein et al., 2005), bird species (Trainer, 1946; Dooling et al., 1971; Maiorana & Schleidt, 1972; Dooling & Saunders, 1975; Hienz et al., 1977; Cohen et al., 1978; Dooling et al., 1979; Saunders & Salvi, 1993; Dooling & Okanoya, 1995; Langemann et al., 1998), as well as a wide range of other animals (for review, see Fay, 1988).

The field of psychophysics is concerned with investigating the relationship between psychological sensation and physical stimuli. One ultimate goal of psychophysics is determining sensory thresholds (Fechner, 1860; Stebbins, 1970; Gescheider, 2013). In psychoacoustic estimation of detection thresholds, the subject is trained to respond with a particular behavior in the presence of a stimulus and in a different manner if the stimulus is absent. The psychophysical studies described in this dissertation were conducted using operant conditioning, a term first coined by B.F. Skinner in 1938, in which the subject receives positive reinforcement (usually a food reward) when it performs a particular behavior in response to the stimulus. Sometimes a secondary reinforcer precedes the food reward, such as a whistle or click, which can be useful to provide immediate feedback, and avoid overfeeding, etc..

Classical conditioning, while more common in past decades, is still used in some experiments (Schusterman, 1980; Fay, 1995; Early et al., 2001; Heffner et al., 2013). In this method, an unconditioned stimulus, such as an electric shock or a puff of air to the eye, is used to elicit a physiological response (a change in respiratory rate or heart rate) or behavioral response (such as an eye-blink). The unconditioned stimulus is paired repeatedly with the acoustic stimulus until the acoustic stimulus alone elicits the measured response.

In a psychoacoustic go/no-go task, which is the method used in this dissertation, the subject is trained to respond with a particular behavior, such as touching an object or producing a sound, in the presence of the stimulus (*go*) and to not respond when the stimulus is absent (*no-go*). This is in contrast to a yes/no task in which the animal is trained to do one task for "yes" and another to "no" (Gerstein et al., 1999; Jensen & Klokker, 2006).

In go/no tasks, the presentation of the stimulus occurs after the animal has stationed at a particular location in a particular orientation (at a button or paddle, in a hoop, at a bite-plate, etc.). Once the stimulus is presented, the subject has a fixed time period to respond (go) with the desired behavior. If no stimulus is presented, the subject remains stationed until the trial has ended (no-go). There are four possible outcomes in this task, shown in Figure 1.7: when a stimulus is present, the subject can either respond correctly ("hit") or fail to respond ("miss"). If the stimulus is absent, the subject can either respond as if there were a stimulus ("false alarm") or not respond ("correct rejection").

		Signal	
		Present	Absent
Response	Go	HIT	FALSE ALARM
	No-Go	MISS	CORRECT REJECTION

Figure 1.7. The four possible responses in a go/no-go task such as the one described in this dissertation. The signal (a tone in this case) is either present or absent on any given trial, and the subject either responds (go) or not (no-go).

There are several widely used methods for presenting the stimuli to the subject. The method used in this dissertation is the modified method of constant stimuli in which the stimulus set (all stimulus values) is pre-selected before testing begins and does not change as a result of the subject's responses (Stebbins, 1970; Dooling & Okanoya, 1995; Kastak & Schusterman, 1998; Wolski et al., 2003). In a detection task, a set number of different stimulus levels are pre-selected and usually presented to the subject in a random order for each block of trials (Figure 1.8).

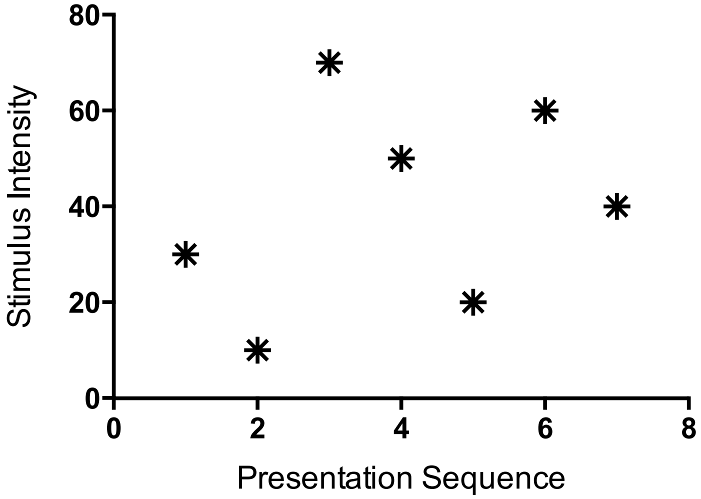

Figure 1.8. The method of constant stimuli. In this example, there are seven individual stimulus presentations, ranging from 10-70 dB re 20 μPa. The presentation order is random in each block.

Threshold is then defined as the stimulus value associated with a specific performance level on stimulus-present trials. This value is usually defined as the stimulus level corresponding to 50% correct detection (Levitt, 1971; Dooling & Okanoya, 1995). The example below (Figure 1.9), taken from Dooling & Okanoya (1995), shows a psychometric function from adult Coturnix quail (*Coturnix japonica*) in a go/no-go psychoacoustic detection task. The stimuli were presented using the method of constant stimuli – the level of the stimulus varied in 10 dB step sizes from 0 to 60 dB re 20 μPa presented in random order. Threshold was then defined as the sound pressure level associated with 50% correct detection on the psychometric function (6.2 dB re 20 μPa).

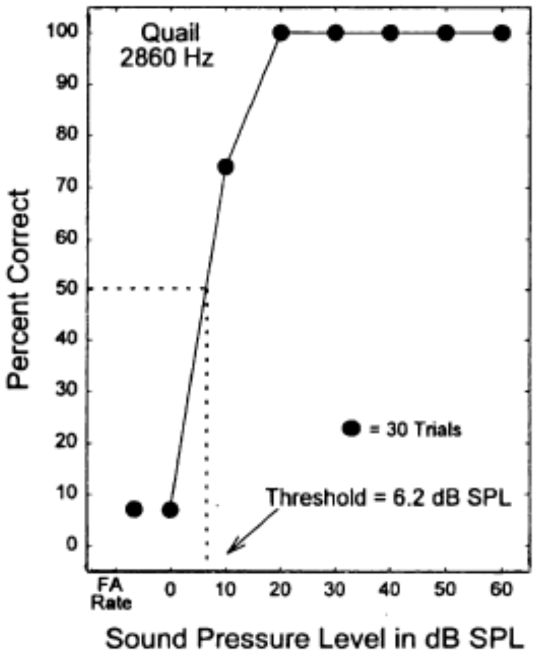

Figure 1.9. A psychometric function from a quail, taken from Dooling & Okanoya (1995). Threshold was defined at the 50% correct level.

The major advantage for using the method of constant stimuli is that the stimuli are presented in random order; and therefore, the subject cannot anticipate future stimulus levels. Thus, any errors of expectation are minimized.

Another method for stimulus presentation is the method of limits, in which the stimuli are presented in small steps in either ascending or descending order, and the subject responds on each trial to the presence or absence of the stimulus (Gescheider, 1997; Syzmanski et al., 1999). Threshold is then estimated as the midpoint between the stimulus steps where the subject changed from a stimulus-present response to a stimulus-absent response. Usually, ascending and descending series are used in alternation. Unlike the method of constant stimuli, threshold is not determined by a statistical probability. However, a disadvantage is that the non-random sequence of stimuli can introduce errors of expectation from the subject.

The up/down staircase method is a variation of the method of limits in which the stimulus values are changed during testing based on the subject's responses (Figure 1.10) (Cornsweet, 1962; Robinson & Watkins, 1973; Gerstein et al., 1999; Wolski et al., 2003). When the animal responds correctly to the presence of a stimulus, the stimulus level is decreased on the next trial, and this decrease continues until the animal fails to respond (miss) to the presence of a stimulus. After this miss, the stimulus level is then increased until the animal makes a correct response, and then decreased again after the correct response until the next miss. This yields a series of reversals, as in Figure 1.10. In this method, the threshold is then defined by averaging the stimulus values across reversal points (Kastak & Schusterman, 1998). This threshold is equivalent to the 50% correct detection threshold obtained with the method of constant stimuli (Levitt, 1970).

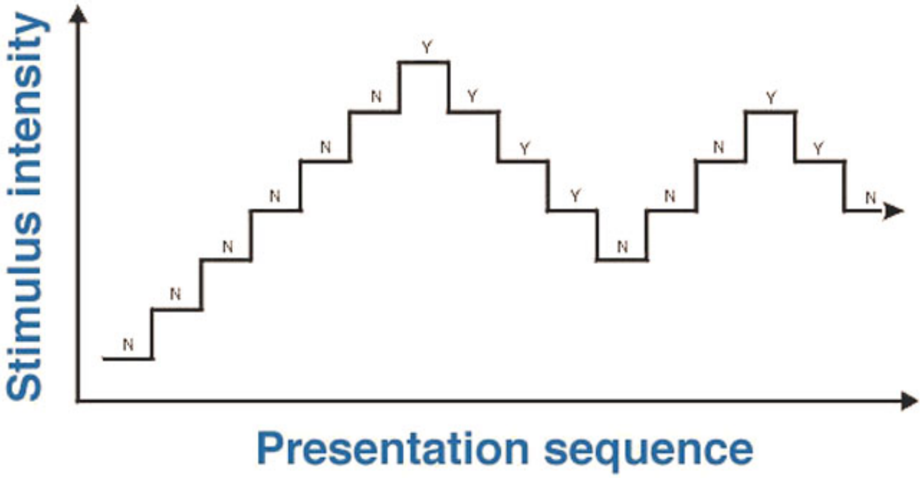

Figure 1.10. The up/down staircase method of stimulus presentation, taken from Kalloniatis & Luu (1995). In this example, a Y = a correct response to the presence of a stimulus, and a N = no response to the presence of a stimulus. The stimuli in this example are presented in ascending order, until a "Y," after which the stimulus is decreased until an "N" occurs, at which time the stimulus is increased again.

The staircase method is efficient because it concentrates only on stimulus intensities near threshold, but a task in which most of the values are close to threshold can be difficult for an animal subject. The subject can also anticipate the approach to threshold, leading to errors similar to the method of limits.

Stimulus detection tasks are sensitive to the non-sensory biases of the animal, such as motivation and expectation (Green & Swets, 1966). Signal detection theory can be used to account for differing probabilities of detection and false alarm rates (Green & Swets, 1966). According to signal detection theory, the sensory evidence that a signal is present falls on a continuum, and the strength of this evidence varies from trial to trial (Ehrenstein & Ehrenstein, 1999). The amount of “noise” also varies across trials, so that even on a trial where there is no stimulus present, there is some evidence to the subject that it might be present. This creates two distributions – a signal + noise distribution when the signal is present, and a noise distribution when a signal is not present (Ehrenstein & Ehrenstein, 1999) (Figure 1.11). The subject sets some criterion, that if exceeded will respond that signal is present (Green, 1960). Using mathematical models, the subject’s responses can be utilized to determine the subject’s criterion and its interaction with the physical properties of the signal (Green, 1960). The variation based on the subject’s criterion can then be removed to leave a more reliable measure of the signal detectability.

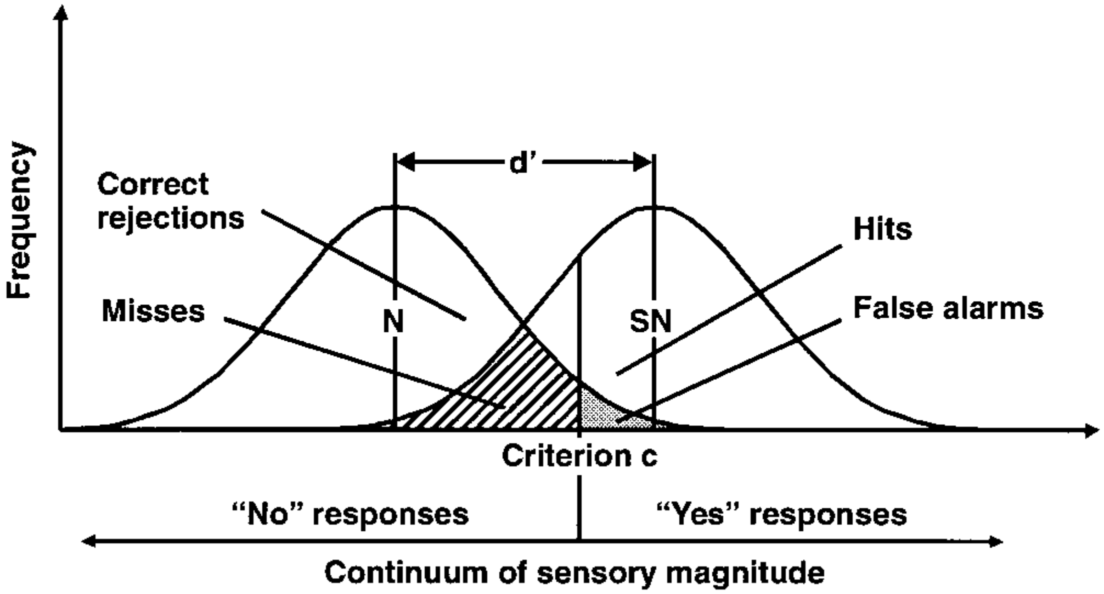

Figure 1.11. Psychophysical distributions of noise (N) and signal + noise (SN), taken from (Ehrenstein & Ehrenstein, 1999). Sensory magnitude, from weak (left) to strong (right) is plotted on the horizontal axis, and sensory excitation on the vertical axis. A subject's theoretical criterion (c) and sensitivity (d) are shown.

One measure of observer sensitivity is d', calculated from the subject's hit and false alarm rates, and defined as the distance between the means of the signal + noise and noise distributions on Figure 1.11 (Swets, 1959; Green & Swets, 1966). This discriminability index is a measure of how well the subject can separate the presence of a signal from noise. The larger the value of d', the larger the separation between signal and noise, with a d' of 0 representing chance-level discrimination (Green & Swets, 1966).

In a psychoacoustic go/no-go task, threshold can be defined at a particular particular d' value instead of at 50% correct detection, using the subject's particular false alarm rate and calculating a new hit rate. For example, Dooling & Okanoya (1995) defined threshold for four quail as 50% correct detection using the method of constant stimuli. They then also defined threshold using signal detection theory, using each bird's false alarm rate and calculating a new hit rate corresponding to given levels of d' (1.0, 1.5, and 2.0) (Figure 1.12). They found that the threshold corresponding to a hit rate of 50% generally tracked a d' of about 1.5 (Figure 1.12).

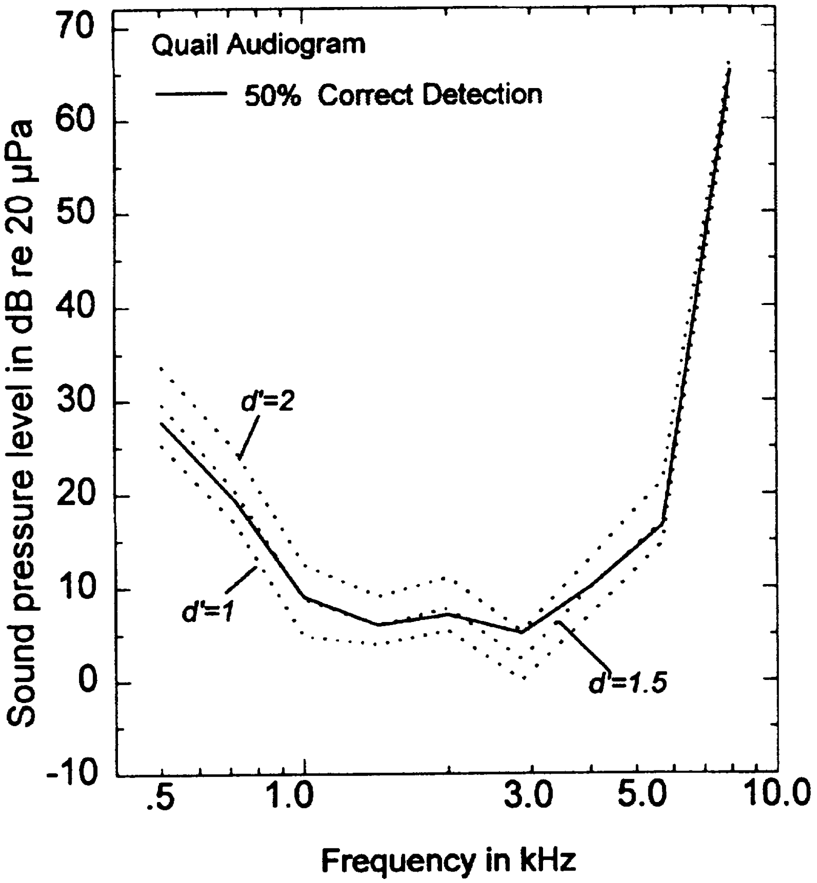

Figure 1.12. Audibility curves for four quail, from Dooling & Okanoya (1995). The solid line represents the average threshold corresponding to a hit rate of 50%. The dashed lines represent thresholds for three levels of d' (1.0, 1.5, and 2.0).

Auditory Brainstem Response

The auditory brainstem response (ABR) can be measured using non-invasive recordings of evoked auditory activity to estimate the auditory sensitivity of an animal. Auditory sensitivity is measured through the evoked potential response of the nervous system along the auditory pathway from the auditory nerve to the auditory brainstem. The ABR is a scalp-recorded potential resulting from neural discharges (population response) synchronized by the onset of a brief acoustic stimulus (Jewett et al., 1970; Jewett & Williston, 1971). The animal's auditory sensitivity may be estimated by measuring changes in the ABR in response to decreasing intensity acoustic stimuli. When the stimulus intensity decreases, the ABR latency increases and the amplitude of the ABR decreases until it is no longer distinguishable from noise, using either visual detection or image recognition algorithms (Hall, 1992).

The ABR is a type of auditory evoked potential that occurs early, (approximately 1-7 ms) following the onset of the stimulus, as opposed to longer latency evoked potentials typically recorded from cortex (Hall, 1992). The response is manifested as a series of waves occurring (Figure 1.13. 1.14). In both birds and mammals, these signals are low-amplitude, up to approximately 10 μV, and only detectable through the averaging of many replications (typically 500-1,000) as well as the removal of large voltage artifacts due to muscle movements (Hall, 1992). The ABRs are generally regarded as the summation of several signals generated in different portions of the auditory system, usually denoted by roman numerals (Hall, 1992).

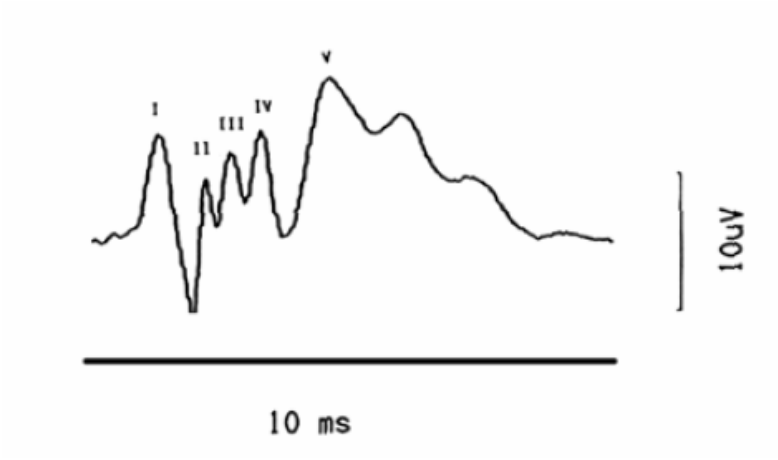

Figure 1.13. The ABR audiogram evoked by a 100 dB SPL click from the cat, taken from Javel, Walsh, & McGee (1986).

The human ABR consists of seven waves, which used to be attributed to seven different structures in the ascending auditory system: Wave 1 – auditory nerve, Wave 2 – cochlear nucleus and trapezoid body, Wave 3 – superior olive complex, Wave 4 – lateral lemniscus, Wave 5 – inferior colliculus, Wave 6 – medial geniculate body of the thalamus, Wave 7 – medial geniculate body (Spehlmann, 1985). This wave-to-point correlation may be too simplistic, however, and structures may contribute to more than one wave (Møller, 1994). Neural correlates for the ABR waves are unknown for bird species, but several studies show that the first peak in bird ABRs may be attributed to the auditory nerve (Katayama, 1985; Brown-Borg et al., 1987; Köppl & Gleich, 2007).

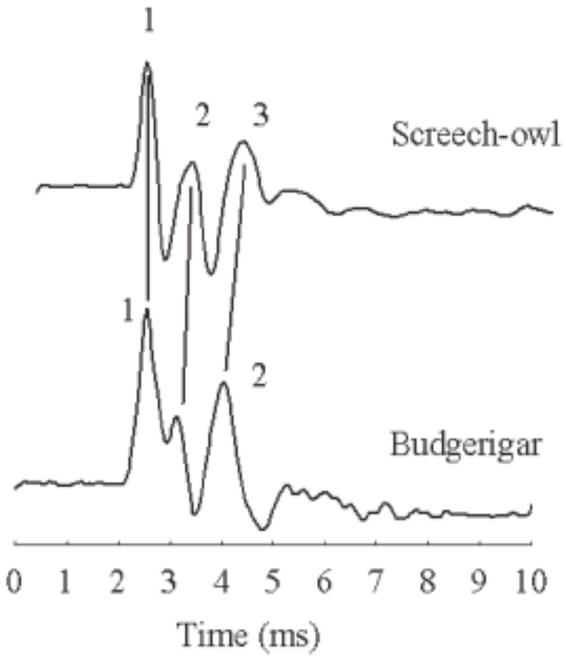

Figure 1.14. Screech owl and budgerigar ABR waveforms evoked by a 100 dB SPL click stimulus (Brittan-Powell et al., 2002, 2005). The third peak in the screech owl waveform most likely corresponds to the second peak in the budgerigar waveform, and the second peak in the owl closely corresponds to the shoulder on wave 1 in the budgerigar.

Thus, the ABR is a useful tool for determining the shape of the audiogram and range of hearing. It should be noted that ABR techniques are not, however, suitable for determining absolute thresholds, i.e. the lowest detection level in a quiet environment, because they rely upon synchronous activation of a population of neurons.

Many studies have directly compared audiograms resulting from psychoacoustics and the ABR and most show a similar pattern. While audiograms obtained from both methods maintain a similar shape, ABR thresholds are usually higher (less sensitive) than psychoacoustic thresholds, as demonstrated with rabbits (*Oryctolagus cuniculus* - Borg & Engström, 1983), rats (*Rattus norvegicus* – Borg, 1982), harbor seals (*Phoca vitulina* – Wolski et al., 2003), bottlenose dolphins (*Tursiops truncatus* – Houser & Finneran, 2006), a false killer whale (*Pseudorca crassidens* – Yuen et al., 2005), several songbird species (Henry & Lucas, 2008), and budgerigars (*Melopsittacus undulatus* – Figure 1.15; Brittan-Powell et al., 2002). The threshold differences range across species and frequencies, but are often 10-15 dB (Gorga et al., 1988). There is some evidence that increased consistency across testing environment (acoustic properties of the room; activity state of subject), stimulus characteristics (duration and bandwidth), presentation method (headphones or free-field sound, placement of speaker), and analysis (averaging, detection method) can minimize these differences, suggesting that the effect is due to both the organism being tested and the procedures used (Szymanski et al., 1999; Schlundt et al, 2007; Ladich & Fay, 2013; Sisneros et al., 2013).

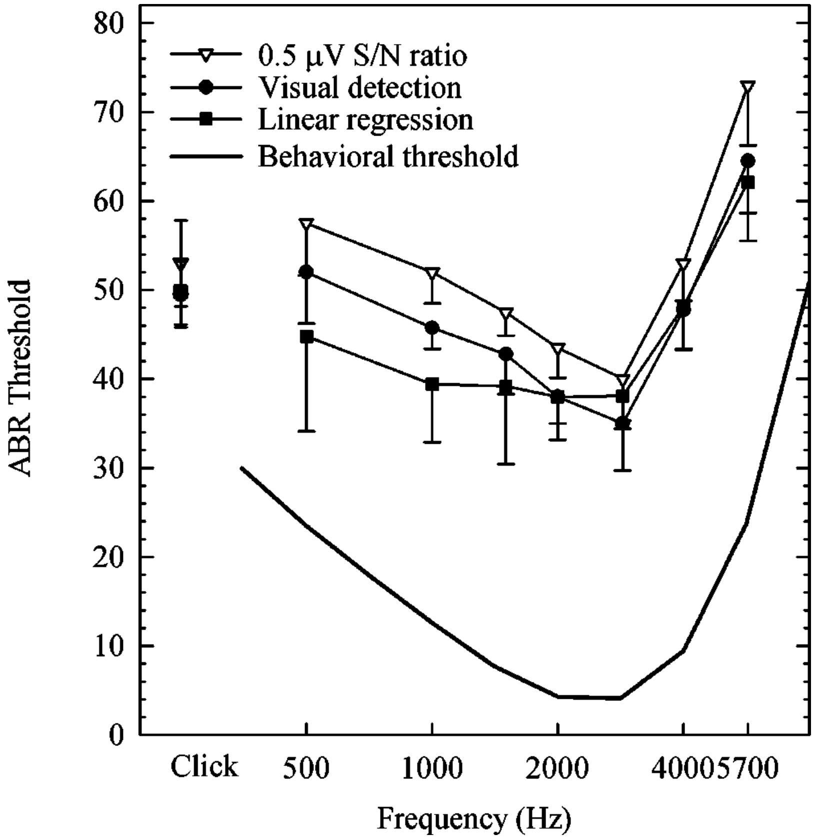

Figure 1.15. Comparison of the budgerigar ABR and behavioral audiogram (Brittan-Powell et al., 2002). ABR audiograms were derived using three methods: 0.5 µV signal/noise ratio (open triangle), visual detection method (closed circle), and the linear regression (closed square). ABR audiograms estimated by all three methods are on average 30 dB higher than the behavioral audiogram.

Stimuli used in ABR studies are short tone bursts (approximately 5 ms) with rapid onsets (approximately 1 ms), while tones used for behavioral methods are much longer (from 250 ms up to several seconds). Psychoacoustic studies usually use longer stimuli because it has been shown that thresholds are higher when the duration of the stimuli is shorter than the integration time of the ear (approximately 200-300 ms for tones in most vertebrates, including birds - Watson & Gengel, 1969; Dooling & Searcy, 1985; Brittan-Powell et al., 2002). Therefore, some of the difference between ABR and behavioral audiograms can attributed to this stimulus length difference. In addition, because ABRs require short stimuli to evoke a synchronized response, such as the onset of a very short tone burst, the auditory system is responding to a wider range of the frequencies around the central stimulus frequency, especially at low frequencies (Silman & Silverman, 1991; Hall, 1992).

The utility of ABR becomes apparent when measuring hearing in an animal that cannot be easily trained for psychoacoustics or kept in captivity for an extended time period. An entire ABR audiogram can often be constructed after one session of approximately 60 min. In comparison, behavioral thresholds involve intensive animal training that can take months to accomplish. Although ABR audiograms may not be good indicators of absolute thresholds, they are useful for estimating a general range of hearing, including the audiogram shape and region of greatest sensitivity (Sisneros et al., 2013). The ABR is also a valuable tool to compare animals tested in the same acoustic setup, or the same individual before and after some kind of intervention, such as noise exposure (Sisneros et al., 2013).

Study Systems Used in this Dissertation

Many studies have already investigated the sensitivity, sound production, and anatomical adaptations of entirely aquatic animals like cetaceans and fish. Amphibious animals face the additional challenge of hearing in more than one medium, and therefore must balance trade-offs of adapting to both air and water. Some diving birds dive to depths comparable to that of pinnipeds, and while considerable attention has been given to pinniped hearing, there are currently no investigations into amphibious hearing in diving birds. Diving birds could use auditory cues to avoid predators, find food, or for auditory scene analysis, the ability to detect, identify and track relevant sounds in the noise of the surrounding environment (Bregman, 1990). While it is a possibility that diving birds communicate with conspecifics under the water, underwater sound production has never been recorded. These possibilities will be discussed in the dissertation discussion (chapter 5).

In-air audiograms already exist for many bird species, and the avian auditory system (as it works in air) has been well studied. Both the electrophysiological and psychoacoustic methods described in this dissertation have been commonly used in the laboratory to examine the avian auditory system. This project includes several species of diving ducks, seaducks, and other aquatic birds. While seaducks do dive, they can be distinguished from diving ducks by their inclusion in a separate subfamily within Anatidae (ducks, swans, and geese) that are essentially marine outside of the breeding season. Table 1.1 lists the common name, scientific name, and the average mass of each species. Some descriptive details on the species follow the table.

Table 1.1. Common name, scientific name, and average mass of each species used in this dissertation.

Common Name	Scientific Name	Average Mass (g)
Black scoter	*Melanitta americana*	1040
Common eider	*Somateria mollisima*	1955
Harlequin duck	*Histrionicus histrionicus*	613
Lesser scaup	*Aythya affinis*	900
Long-tailed duck	*Clangula hyemalis*	750
Northern gannet	*Morus bassanus*	3000
Red-throated loon	*Gavia stellata*	1850
Ruddy duck	*Oxyura jamaicensis*	564
Surf scoter	*Melanitta perspicillata*	975
White-winged scoter	*Melanitta fusca*	1370

Lesser scaup (*Aythya affinis*) are a medium-sized diving duck that feeds primarily on mollusks, crustaceans, and aquatic insects. They are capable of diving to depths of at least 15-18 m, for 2-25 seconds at a time. Both males and females vocalize throughout the year to signal to mates and offspring. The lesser scaup is one of the most abundant and widespread species of diving duck, but its numbers have been declining in recent years for unknown reasons (Austin et al., 1998).

Long-tailed ducks (*Clangula hyemalis*) are the deepest divers of all diving and seaducks, reaching at least 60 m of depth to search for crustaceans, fish, and mollusks. They are also arguably the most vocal of the seaducks, with a distinctive and often incessant *ow-owoolee* male call. They are a true Arctic species, breeding in tundra and taiga regions (Robertson & Savard, 2002).

Surf scoters (*Melanitta perspicillata*) are a seaduck species that dive mainly for mollusks, with a mean dive duration of approximately 30 seconds. Generally silent, the male can make a gurgling call during courtship and the females a crow-like call when defending ducklings (Savard et al., 1998).

White-winged scoters (*Melanitta fusca*) are the largest of all the scoters, and like the surf scoters are not very vocal. Females will protect the nest and ducklings with a whistle-like call (Brown & Fredrickson, 1997).

Black scoters (*Melanitta americana*) are the least studied of all scoters. They primarily forage on mollusks and aquatic insects. They are the most vocal of all scoters, with the males continuously emitting a frequency-modulated melodious whistle (Bordage & Savard, 2011).

Harlequin ducks (*Histrionicus histrionicus*), another seaduck species, spend the breeding season in clear, fast-moving rivers where they forage for larval insects and small crustaceans. Given their nickname of “sea mice,” harlequins produce a constant mouse-like squeak during courtship, agonistic interactions, and calls to ducklings (Robertson & Goudie, 1999).

Ruddy ducks (*Oxyura jamaicensis*) feed primarily on midge larvae and are distinct among all diving ducks, because of their unique courtship behavior, which consists of slapping their bill on their chest and producing a “belching” sound. These ducks are generally silent, except for a high-pitched peep and the courtship sound. They are also extremely aquatic, with legs set so far back on their bodies that walking on land is difficult (Brua, 2002).

Common eiders (*Somateria mollisima*) are the largest duck found in the northern hemisphere and can weigh up to 3040 g. These birds are highly adapted for life in frigid waters. Their calls are hoarse, grating and cooing sounds (Goudie et al., 2000).

Red-throated loons *(Gavia stellata)* are the smallest members of the loon family (Gavidae). They pursue live fish underwater, including herring (Clupeidae), capelin (Mallotus villosus) and sculpin (superfamily Cottoidea). They do not "yodel" like other loon species, but instead use their "plesiosaur call" as a territorial duet (Barr et al., 2000).

Northern gannets *(Morus bassanus)* are the largest indigenous seabirds in the North Atlantic, belonging to the family Sulidae (boobies and gannets). They obtain live fish, mostly mackerel (Scombridae) and herring, through plunge diving, during which the bird starts from a height of 10-40m above the water and plunges into the water with speeds >100 km/hr. They then pursue fish up to 15 m deep in the water by swimming. They breed in dense, noisy colonies on cliffs or islands (Mowbray, 2002).

Dissertation Outline

In this dissertation, I have used behavioral and electrophysiological methods to measure in-air and underwater auditory sensitivity in aquatic birds. In chapter 2, I used the auditory brainstem response to measure in-air auditory sensitivity in ten species of aquatic birds. In chapter 3, I measured in-air thresholds in one species of diving duck, the lesser scaup, using both psychoacoustics and the ABR. In chapter 4, I measured in-air and underwater sensitivity in a species of seaduck, the long-tailed duck, using psychoacoustics. Chapter 5 summarizes these findings and their implications.

Chapter 2: A Comparison of Auditory Brainstem Responses across Aquatic Bird Species

INTRODUCTION

Hearing abilities have been measured in only approximately 50 of the 10,000 species of extant birds (Dooling, 2000, 2003). Of these 50 species, only two are considered aquatic– the black-footed penguin (*Spheniscus demersus*; Wever et al., 1969), and the mallard duck (*Anas platyrhynchos*; Trainer, 1946). These two species are from different taxonomic families, and vary in the habitat they occupy (penguins are exclusively marine and mallards are found throughout coastal and freshwater waterways), their aquatic lifestyle (penguins are adapted for swimming underwater and mallards live at the water's surface), their social structure (penguins nest in dense colonies with males and females both incubating the eggs, while mallard nests are scattered throughout a range of environments and only females care for young), and foraging habits (penguins pursue live fish, while mallards eat grasses, seeds, and invertebrates). The divergence between these two species makes it difficult to determine if generalizations about aquatic bird hearing are possible, therefore, there is a fundamental need to extend our knowledge of hearing capabilities to other aquatic bird species to allow for potential phylogenetic, physiological, and life history comparisons.

Hearing in aquatic bird species may be impacted by general adaptations for living in an aquatic environment. Birds that dive more than a few meters may have adaptations to compensate for increasing water pressure on internal air spaces, such as the air-filled middle ear. Aquatic mammals may provide examples of how ear anatomy is adapted for diving. For example, the ear anatomy of the pinnipeds (seals, sea lions, and the walrus) has been adapted for diving in several ways. There is muscular control of the meatal opening to prevent water from entering the meatus, which is very narrow and waxy (Rampreashad et al., 1972; Kastalein, 1996; Welsch & Riedelsheimer, 1997; Stenfors et al., 2000). In addition, the meatus and middle ear are lined with cavernous tissue, which is highly vascularized and fills with blood to compensate for increasing pressure on the tympanic membrane as the animal dives (Repenning, 1972; Stenfors et al., 2000).

Although ear anatomy in aquatic birds is not well-investigated, some penguin species have similar adaptations for diving to those found in mammals, such as the cavernous tissue in the meatus and middle ear and active muscular control of the meatal opening (Sade, 2008). In addition, aquatic birds have specialized feather structure that creates a waterproof outer covering, including over the meatal opening (Rijke, 1970). Any of these adaptations for diving could possibly impact auditory sensitivity in the air by changing tissue impedances and structural shape. For example, interlocking feathers over the meatus for waterproofing could impede sensitivity in the air by creating a sound barrier.

Aquatic bird families are scattered throughout the avian phylogeny and it is assumed that the aquatic lifestyle did not evolve from a common ancestor. Diving abilities range greatly across aquatic bird families – with the diving ducks at the shallow end of the continuum (tens of meters) and the penguins at the other end (greater than 500 m) (Roberston et al., 2002; Meir, 2008). Habitats for different species range from inland ponds and lakes to open ocean. Many aquatic bird species, especially marine-oriented seabirds (such as penguins, gannets, albatross, and auks) are colonial nesters, with thousands of nesting birds in one small area. Other aquatic bird species, such as the ducks or waterfowl, have low densities of nests scattered across a wide geographical area. These extensive differences in where birds live may have influenced sensory biology and it is important to examine auditory sensitivity across aquatic bird species that evolved separate adaptations to life on the water.

In addition to developing an understanding of aquatic bird hearing to compare to non-aquatic birds, such knowledge would also provide valuable information relevant to management issues, such as the introduction of man-made noise into flyways, critical stopover points during migration, or breeding areas. Aquatic birds are exposed to a variety of man-made noise sources, depending on their habitat. Species that occupy inland freshwater bodies, like some duck species, are exposed to noises typical in populated areas, such as traffic noise. Coastal birds are potentially impacted from sources such as recreational boating, commercial shipping, and coastal construction. Aquatic birds living farther from the coast could be most exposed to noise from commercial shipping and offshore energy development.

Increased noise levels in a bird’s habitat have the potential to cause a bird to alter its communication signals, mask communication signals or other biologically relevant sounds, cause avoidance of particular areas, decrease reproductive success, and increase physiological stress (Reijnen et al., 1996; Campo et al, 2005; Dooling & Popper, 2007; Blickley et al., 2012; McClure et al., 2013; Naguib et al., 2013; Slabbekoorn, 2013).

Given the lack of information available, it is important to conduct a comprehensive investigation into aquatic bird hearing. Electrophysiological and behavioral methods are commonly used in the laboratory to examine the avian auditory system. Behavioral audiograms generally produce thresholds that are more sensitive than those obtained using the ABR, but these studies require months of animal training, and work best with animals in captivity (Borg, 1982; Borg & Engström, 1983; Gorga et al., 1988; Brittan-Powell et al., 2002; Wolski et al., 2003; Yuen et al., 2005; Houser & Finneran, 2006; Henry & Lucas, 2008). Application of a time-efficient, minimally invasive technique such as the auditory brainstem response (ABR), can be a valuable physiological method to test hearing in wild aquatic bird species. The ABR allows us to explore the auditory system more rapidly than behavioral techniques, in as little as one hour, and on wild-caught birds.

The ABR has been used as a tool for studying the functionality of the auditory system in a wide variety of animals, including several species of birds, such as budgerigars (*Melopsittacus undulatus*), screech owls (*Megascops asio*), several woodpecker species, and red-winged blackbirds (*Agelaius phoeniceus*) (Brittan-Powell et al., 2002, 2005; Henry & Lucas, 2010; Lohr et al., 2013). The ABR is a scalp-recorded potential resulting from synchronized neural discharge (population response), manifested as a series of four or more waves occurring within the first 10 ms following stimulation and representing the progressive propagation of auditory neural activity through the ascending auditory pathway (Katayama, 1985; Hall, 1992; Brittan-Powell et al., 2002).

The goal of this study was to evaluate the auditory abilities of a variety of aquatic birds to extend knowledge of bird hearing to aquatic species, and provide a baseline to facilitate future management actions concerning the introduction of noise into aquatic bird habitats. Objectives included: 1) comparing hearing sensitivity across various aquatic bird species using ABR, 2) evaluate the effects of different anesthetics on the ABR, and 3) investigate correlations between hearing sensitivity and vocalization characteristics for each species.

METHODS

The Animal Care and Use Committees at both the University of Maryland and the U.S. Geological Survey Patuxent Wildlife Research Center (where the birds were housed and tested) approved all of the following procedures.

Subjects

This study included ten species of birds, with three to ten individuals tested per species, based on availability (Table 2.1). The majority of the species tested were seaducks and diving ducks. While seaducks do dive, they can be distinguished from diving ducks by their inclusion in a separate subfamily within Anatidae (the waterfowl: ducks, swans, and geese) that are essentially marine outside of the breeding season. Descriptive details for each of the species follow (as duplicated from Chapter 1):

Lesser scaup (*Aythya affinis*) are a medium-sized diving duck that feeds primarily on mollusks, crustaceans, and aquatic insects. They are capable of diving to depths of at least 15-18 m, for 2-25 seconds at a time. Both males and females vocalize throughout the year to signal to mates and offspring. The lesser scaup is one of the most abundant and widespread species of diving duck, but its numbers have been declining in recent years for unknown reasons (Austin et al., 1998).

Long-tailed ducks (*Clangula hyemalis*) are the deepest divers of all diving and seaducks, reaching at least 60 m of depth to search for crustaceans, fishes, and mollusks. Also long-tailed ducks may be the most vocal of the seaducks, having a distinctive and often incessant *ow-owoolee* male call. They are a true Arctic species, breeding in tundra and taiga regions (Robertson & Savard, 2002).

Surf scoters (*Melanitta perspicillata*) are a seaduck species that dive mainly for mollusks, with a mean dive duration of approximately 30 seconds. Generally silent, the male can make a gurgling call during courtship and the females a crow-like call when defending ducklings (Savard et al., 1998).

White-winged scoters (*Melanitta fusca*) are the largest of all the scoters, and like the surf scoters, are not very vocal. Females protect the nest and ducklings with a whistle-like call (Brown & Fredrickson, 1997).

Black scoters (*Melanitta americana*) are the least studied of all scoters. They primarily forage on mollusks and aquatic insects. They are the most vocal of all scoters, with the males continuously emitting a frequency-modulated melodious whistle (Bordage & Savard, 2011).

Harlequin ducks (*Histrionicus histrionicus*), another seaduck species, spend the breeding season in clear, fast-moving rivers where they forage for larval insects and small crustaceans. Given their nickname of "sea mice," harlequins produce a constant mouse-like squeak during courtship, agonistic interactions, and calls to ducklings (Robertson & Goudie, 1999).

Ruddy ducks (*Oxyura jamaicensis*) feed primarily on midge larvae and are distinct among all diving ducks, because of their unique courtship behavior, which consists of slapping their bill on their chest and producing a "belching" sound. These ducks are generally silent, except for a high-pitched peep and the courtship sound. They are also extremely aquatic, with legs set so far back on their bodies that walking on land is difficult (Brua, 2002).

Common eiders (*Somateria mollisima*) are the largest duck found in the northern hemisphere and can weigh up to 3040 g. These birds are highly adapted for life in frigid waters. Their calls are hoarse, grating and cooing sounds (Goudie et al., 2000).

Red-throated loons *(Gavia stellata)* are the smallest members of the loon family (Gavidae). They pursue live fish underwater, including herring (Clupeidae), capelin (Mallotus villosus) and sculpin (superfamily Cottoidea). They do not "yodel" like other loon species, but instead use their "plesiosaur call" as a territorial duet (Barr et al., 2000).

Northern gannets *(Morus bassanus)* are the largest indigenous seabirds in the North Atlantic, belonging to the family Sulidae (boobies and gannets). They catch live fish, mostly mackerel (Scombridae) and herring, through plunge diving, during which the bird starts from a height of 10-40m above the water and plunges into the water with speeds >100 km/hr. They then pursue fish up to 15 m deep in the water by swimming. They breed in dense, noisy colonies on cliffs or islands (Mowbray, 2002).

Table 2.1 Common name, scientific name, number of individuals, whether they were captive or wild-caught, and the average mass for the specimens of each species used in this study.

Common Name	Scientific Name	Number	Captive or Wild-Caught	Average Mass (g)
Black scoter	*Melanitta americana*	3	Captive	1040
Common eider	*Somateria mollisima*	10	Wild-Caught	1955
Harlequin duck	*Histrionicus histrionicus*	7	Captive	613
Lesser scaup	*Aythya affinis*	6	Captive	900
Long-tailed duck	*Clangula hyemalis*	7	Wild-Caught	750
Northern gannet	*Morus bassanus*	7	Wild-Caught	3000
Red-throated loon	*Gavia stellata*	6	Wild-Caught	1850
Ruddy duck	*Oxyura jamaicensis*	6	Captive	564
Surf scoter	*Melanitta perspicillata*	9	Wild-Caught	975
White-winged scoter	*Melanitta fusca*	6	Captive	1370

Subjects were all adult birds of both sexes, as determined by either captive history or plumage patterns. Captive subjects were raised from eggs at U.S. Geological Survey Patuxent Wildlife Research Center in Laurel, Maryland. Wild subjects were caught as part of an on-going satellite telemetry study (Bureau of Ocean Energy and Management, 2013; Sea Duck Joint Venture, 2012) from areas along the mid-Atlantic and New England coastline, transported to a veterinary hospital for testing, banding, transmitter attachment, and then released. ABR testing occurred before transmitter attachment surgery.

Experimental Procedures

All subjects, whether wild-captured or captive, were tested using the same procedures and equipment, in a veterinary hospital. Birds were sedated with isoflurane (5% for induction, 2-4% for maintenance with oxygen at 1L/min/kg; the lowest possible percentage of isoflurane was used to prevent movement in the bird) prior to electrode placement. A mask was used to induce isoflurane anesthesia, and the bird was intubated once motionless. Electrodes were placed once the bird was motionless for several minutes. Body temperature was monitored with a Cooper-Atkins Electro-Therm thermistor probe (Model TM99A; Middlefield, CT), and remained between 38-40°C. The bird was positioned, on a table, so that the speaker (Pioneer B11EC80-02F 5-1/4"; Longbeach, CA; frequency response 320 - 6000 Hz) was 20 cm from the bird's right ear (Figure 2.1).

Figure 2.1. Top: An intubated surf scoter undergoing an ABR test, showing electrode and speaker placement. Bottom: Electrode placement on an intubated black scoter. Three electrodes were placed subdermally high on the bird's forehead (active), directly behind the right ear canal (the ear ipsilateral to the speaker, reference), and behind the canal of the ear contralateral to stimulation (ground).

Stimuli

Subjects were presented with stimuli made up of tone bursts of 5 ms duration (1 ms rise/fall time and 3 ms steady-state) and 20 ms interstimulus intervals. Tone bursts frequencies ranged from 500 – 5700 Hz and intensities from 30-90 dB re 20 μPa. Each stimulus set was comprised of a train of nine single frequency tone bursts that increased successively in intensity and were presented at a rate of 4/s (Figure 2.2; see Brittan-Powell et al., 2002; 2005; 2010). Stimuli within each train increased in 5-dB steps from 35-55 dB, then in 10-dB steps from 60-90 dB. Sound frequencies were presented in ascending order. In addition to the tone bursts, click stimuli were presented to the bird at a constant intensity (80 dB re 20 µPa) and with a repetition rate of 20/s, at the beginning of data collection and at the end to determine if ABR amplitude and latency changed over the course of the trial (due to anesthesia, physiological state, etc.). This additional click test was conducted only on those birds that were not going into surgery for satellite transmitter implantation after the ABR (n=28; all captive birds listed in table 2.1) in order to minimize the time that these birds were anesthetized.

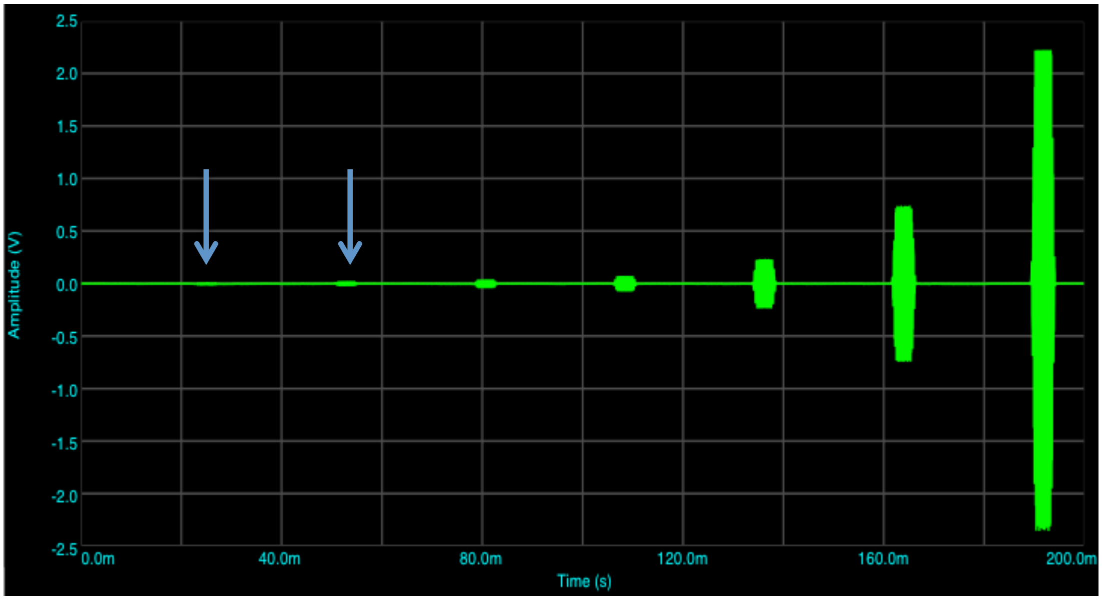

Figure 2.2. A sample stimulus train, with 7 of the 9 tone bursts displayed on an oscilloscope screen. It was not possible to display all 9 tone bursts simultaneously because of scaling – the two highest amplitude stimuli are not in the recording. Arrows point to the two lowest amplitude stimuli. These stimulus voltages were recorded at the input to the speaker.

Recording Equipment and Procedure

Three standard platinum alloy needle electrodes (Grass F-E2; West Warwick, RI) were placed subdermally high on the bird's forehead (active), directly behind the right ear canal (the ear ipsilateral to the speaker, reference), and behind the canal of the ear contralateral to stimulation (ground), (Figure 2.1) as in Brittan-Powell et al. (2002; 2005; 2010). Shielded electrode leads were twisted together to reduce electrical noise through common-mode rejection.

The stimulus presentation and ABR acquisition were synchronized using a Tucker-Davis Technologies (TDT; Gainesville, FL, USA) mobile real-time processor (RM2) controlled by a Gateway PC (Irvine, CA) (Figure 2.3). Sound stimulus waveforms were generated using OpenABR software (developed by Dr. Edward Smith, University of Maryland) and fed to the RM2 for D/A conversion, and then through an amplifier (Pyle PLMRMP1A; Brooklyn, NY) to drive the speaker. The electrodes were connected to a TDT RA4LI headstage and RA4PA Medusa preamplifier that amplified at 20X gain and digitized the signal before sending it over fiber optic cables to the TDT RM2, after which they were analyzed using OpenABR.

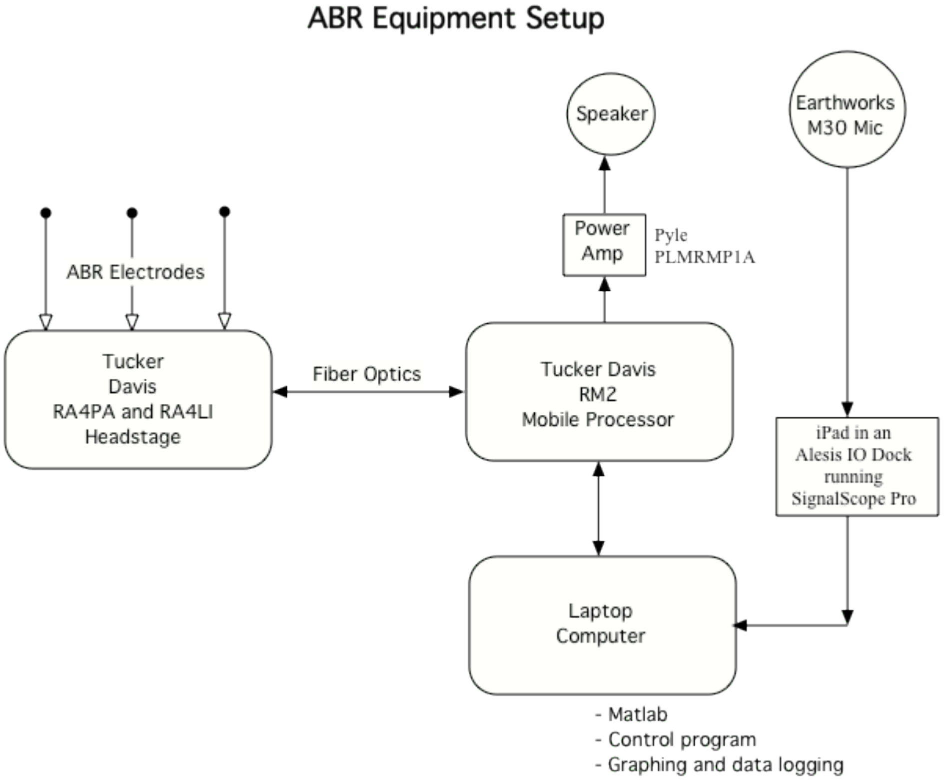

Figure 2.3. Diagram of ABR equipment set-up.

Each ABR represents the average response of 600 stimulus train presentations (alternating polarity/phase to cancel the cochlear microphonic), sampled at 20 kHz for 235 ms following onset of the stimulus. This allowed for 25 ms recording time for each stimulus. The biological signal was amplified and notch filtered at 60 Hz with the OpenABR software. The signal was bandpass filtered between 30 Hz and 3,000 Hz after collection using ABRomatic software (also developed by Dr. Edward Smith, University of Maryland).

Stimulus intensities were calibrated in the free field by placing a ¼” microphone (Earthworks M30-Calibrated; Milford, NH) at the approximate position of the animal’s ear (20 cm from the speaker). The microphone was connected to an iPad in an Alesis IO Dock (Cumberland, RI) running Signal Scope Pro software SPL module (Faber Acoustical; Santaquin, UT) that displayed the sound pressure level of calibration tones. The microphone and Signal Scope software were calibrated prior to each testing session by playing a known SPL tone through the system with a CEM 5C-05 calibrator (Shenzhen, China). Calibration through the OpenABR software consisted of playbacks of one-second tones which were then measured using the fast-weighting flat setting in Signal Scope and the dB levels were entered back into OpenABR for adjustment.

At the end of the experiment, the electrodes were removed. Birds remained isolated in a crate and monitored until they showed normal alertness (head held upright, eyes remaining open, normal preening behavior) returned (usually 1-2 hours). Birds were then returned to the captive flock or released at the capture location. Captive birds were checked throughout the next day (identified by unique leg bands) to ensure good health and recovery. The health of the wild birds was monitored using a body temperature sensor incorporated into the implanted telemetry device. No morbidity or mortality was found associated with the ABR testing.

Statistical analyses were performed using GraphPad Prism statistical software (GraphPad Software, Inc., La Jolla, 2013). All statistical tests were considered significant at the 5% level.

Latency and Amplitude:

The amplitude and latency of the first peak of the ABR was measured for all stimulus frequencies and intensities tested (Figure 2.4). The latency was corrected for the acoustic delay between the speaker and the bird's ear (0.59 ms). The amplitude of the first peak was determined by averaging the section of the waveform before the response began (0-1.5 ms after the stimulus was played) and subtracting this average from the peak (peak to baseline measurement).

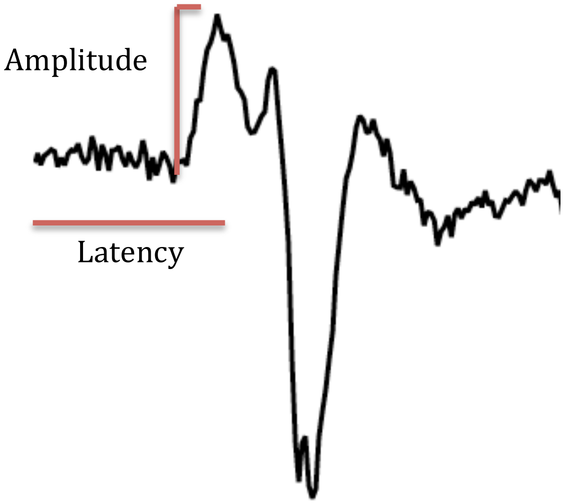

Figure 2.4. Amplitude and latency measurements on a lesser scaup ABR. Amplitude of the first peak was measured as peak to baseline. Latency of the first peak was corrected for the delay between the speaker and the bird's ear.

Threshold Estimation

Threshold was defined using two methods: visual detection and linear regression. In the visual detection technique, the first 10ms of all ABR waveforms was examined visually by observers (who had no prior experience analyzing ABR data) for a response. These observers were trained to identify threshold as the level one half step below the lowest stimulus level at which a response could be visually detected on the trace (as in Brittan-Powell & Dooling, 2004; Brittan-Powell et al., 2005, 2010; Lohr et al., 2013). To test if the observers varied in their analysis, 40 files were chosen at random and analyzed by both observers. A paired t-test was conducted to look for significant differences in thresholds across observers.

Thresholds were also estimated using linear regression analysis on lesser scaup data. The amplitude of the first positive peak was obtained across all frequency and stimulus levels and an amplitude-intensity function was generated. Threshold was defined as the 0 μV crossing of a line produced with linear regression. Techniques for estimating thresholds (visual detection vs. linear regression) within one species (six female lesser scaup) were evaluated, using repeated measures ANOVA.

To compare measures across species and frequencies, repeated-measures two-way analysis of variances (ANOVAs) were conducted. Differences between sexes were not tested due to limited power to detect differences from small sample sizes within sexes for each species.

To investigate the relationship between body mass and best frequency of hearing, the audiogram points for each species were used to calculate a best-fit third-order polynomial in 100-Hz frequency steps for the range of frequencies tested (as in Gleich et al., 2005). The frequency of best hearing (i.e., the frequency with the lowest threshold) and the high-frequency limit of hearing (defined as the point on the high-frequency side of the audiogram where the threshold rises to >30 dB above the lowest threshold) were determined from these functions. Linear regression was used to investigate relationships between body mass, frequency of best hearing, and high-frequency limit of hearing.

Anesthesia Comparisons

Isoflurane was chosen as the anesthetic for these experiments because of its reputation of reliability and safety in waterfowl (Machin, 2004; Carpenter, 2013). Experiments on four additional lesser scaup were conducted to compare ABR results between two types of anesthesia,: isoflurane vs. a combination of ketamine and midazolam (Machin & Caulkett, 1998; Carpenter, 2013). Each duck received both treatments, with order of anesthetic determined by a randomized schedule and with a minimum of two weeks between treatments for a washout period (time for the anesthetic to be eliminated from the animal's system). All equipment and stimulus procedures were as previously described except for ketamine/midazolam delivery, which required a single intramuscular injection of ketamine (40 mg/kg) and midazolam (2 mg/kg) to produce a sufficient level of anesthesia similar to the isoflurane for a period (usually 20-30 min) long enough to complete the ABR trial.

Vocalization Analysis

Vocalizations from eight of the ten species were obtained from Cornell University's Macaulay Library collection. It was not possible to obtain vocalizations from surf scoters or white-winged scoters (neither of which are very vocal (*personal observation*)). Spectrographic analysis of minimum, maximum, and peak frequency (the frequency of the greatest power) was performed on ten individual calls of each species using Raven Lite 1.0 (Cornell Lab of Ornithology; Ithaca, New York). These values were then compared to the most sensitive hearing frequency, which was calculated as described above for the body mass analysis.

RESULTS

All species tested showed at least two prominent ABR peaks within 4-5 ms after the stimulus reached the bird's ear canal. Waveform morphology was very similar across all eight duck species tested (Figure 2.5A), with a different pattern of peaks in the two non-duck species, the red-throated loon and northern gannet (Figure 2.5B). These peak patterns were stable across frequencies and intensity levels. As the level of stimulation increased, ABR amplitudes increased and peak latencies decreased (Figure 2.6).

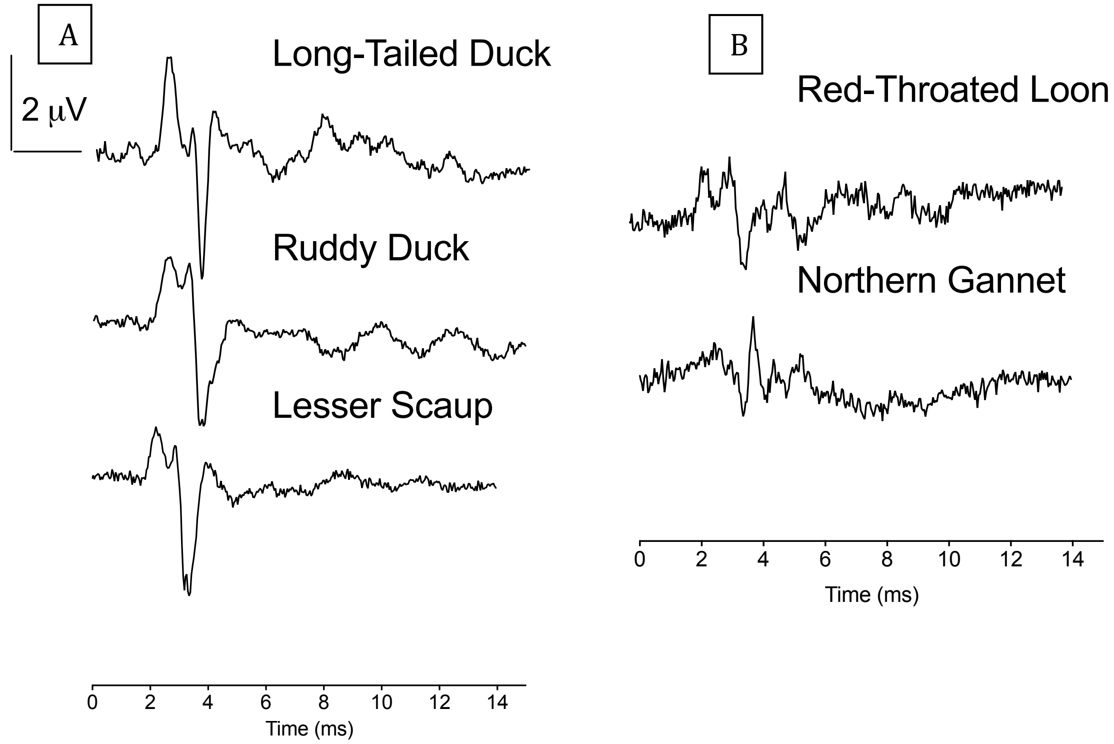

Figure 2.5. Waveform morphology examples from three duck species (A) and two non-duck seabirds (B). All waveforms are responses to 90 dB tone pips at 2860 Hz, the frequency at which the highest amplitude responses were recorded for most species. The stimulus was presented at time=0.

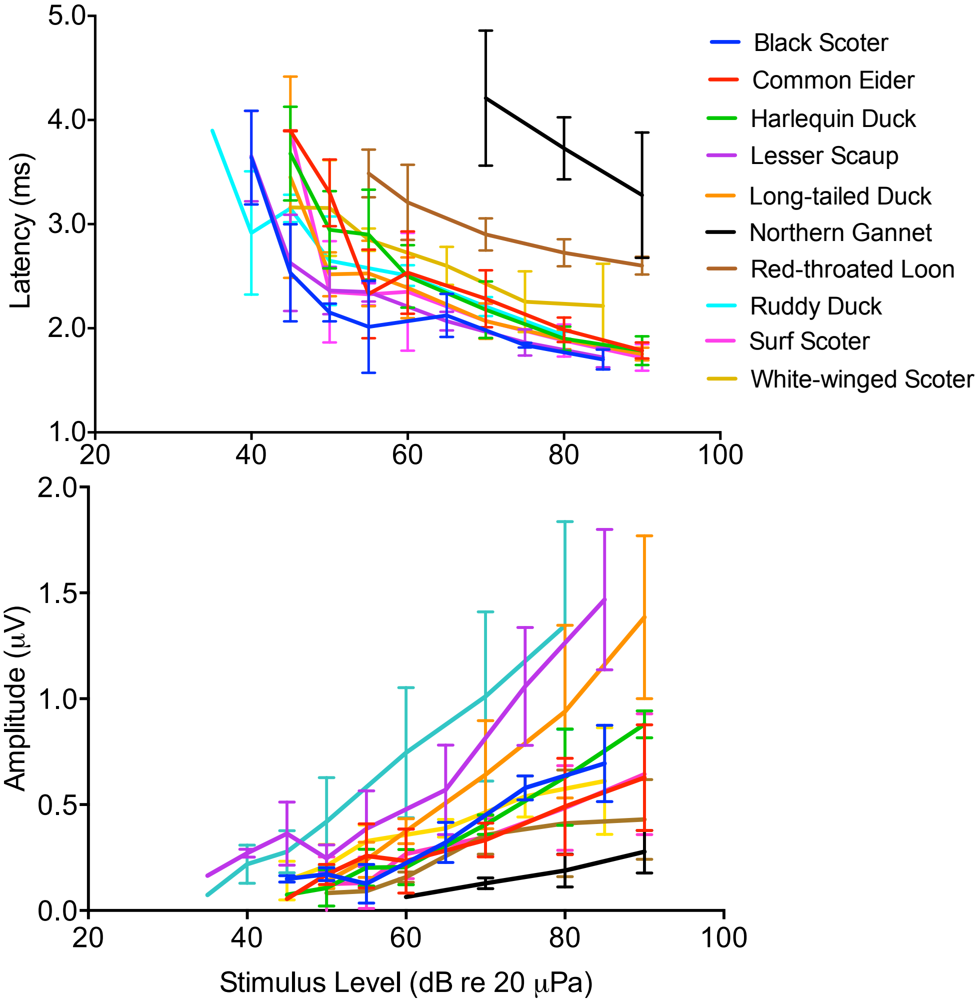

Figure 2.6. The latency (top) and amplitude (bottom) of the first peak as a function of increasing stimulus level at 2860 Hz, the frequency at which the highest amplitude responses were recorded for most species. Latencies are corrected for the delay from the speaker to the bird's ear canal. Vertical bars represent +/- one standard deviation.

The measured audiograms for all birds were U-shaped (Figure 2.7). Sensitivity peaked between 1000-3000 Hz, with a steep high-frequency roll-off after 4000 Hz. A two-way repeated measures ANOVA found significant effects of frequency ($F_{(5,225)} = 114.4$, $p < 0.0001$), species ($F_{(7,45)} = 7.281$, $p < 0.0001$) and frequency by species interaction ($F_{(35,225)} = 2.165$, $P = 0.0004$). There were differences in average audiograms across species (Figure 2.7) with an apparent segregation occurring between waterfowl species and non-waterfowl species. The highest thresholds were found in the northern gannet and red-throated loon and the lowest to the lesser scaup and ruddy duck (Figure 2.8). At the lower frequencies the harlequin duck, common eider, and white-winged scoter exhibited similar thresholds as the two non-waterfowl species, the red-throated loon and northern gannet. However, as the frequencies increased, all three species diverged away from the non-waterfowl species and resembled more closely the other waterfowl species thresholds. Within the waterfowl species, the common eider showed the highest thresholds across all frequencies closely followed by the harlequin duck and the white-winged scoter. At the highest frequency, all species except the lesser scaup converged to a similar threshold around 80 dB re 20 μPa.

Threshold estimates for six female lesser scaup did not differ between the visual inspection method and the linear regression method across frequencies ($F(_{1,8}) = 2.524$, $p = 0.15$; Figure 2.9). Thresholds also did not differ significantly between visual observers ($t=1.38$, $df = 39$, $p = 0.18$). The amplitude and latency of clicks from the 28 captive birds tested did not differ from the beginning to the end of a testing session (amplitude: $t = 0.4786$, $df = 27$, $p = 0.6361$; latency: $t = 1.980$, $df = 27$, $p = 0.0616$).

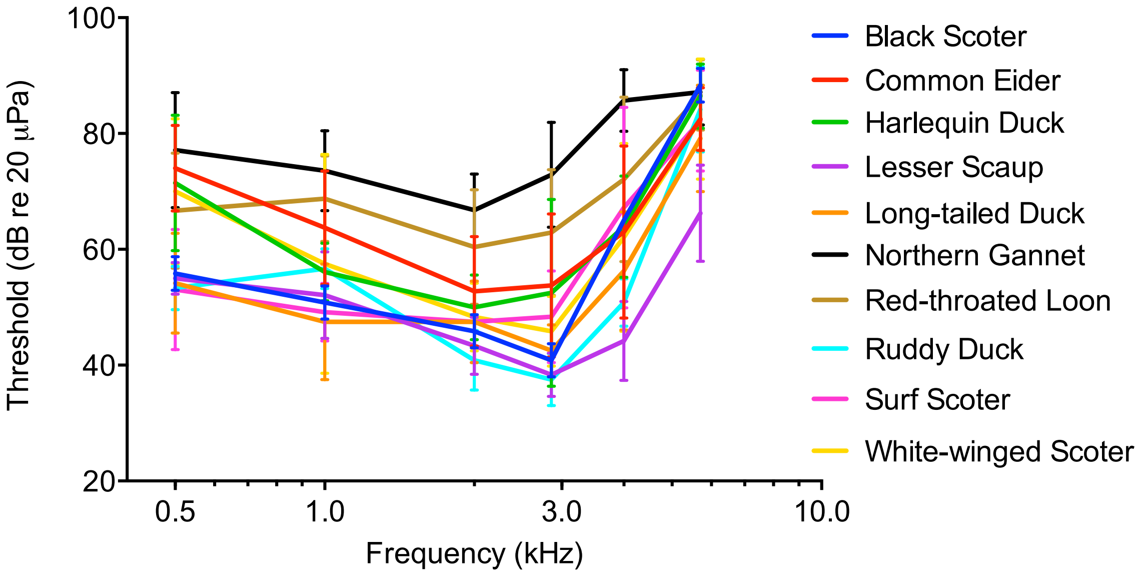

Figure 2.7. Average ABR audiograms from all species tested. Vertical bars represent +/- one standard deviation.

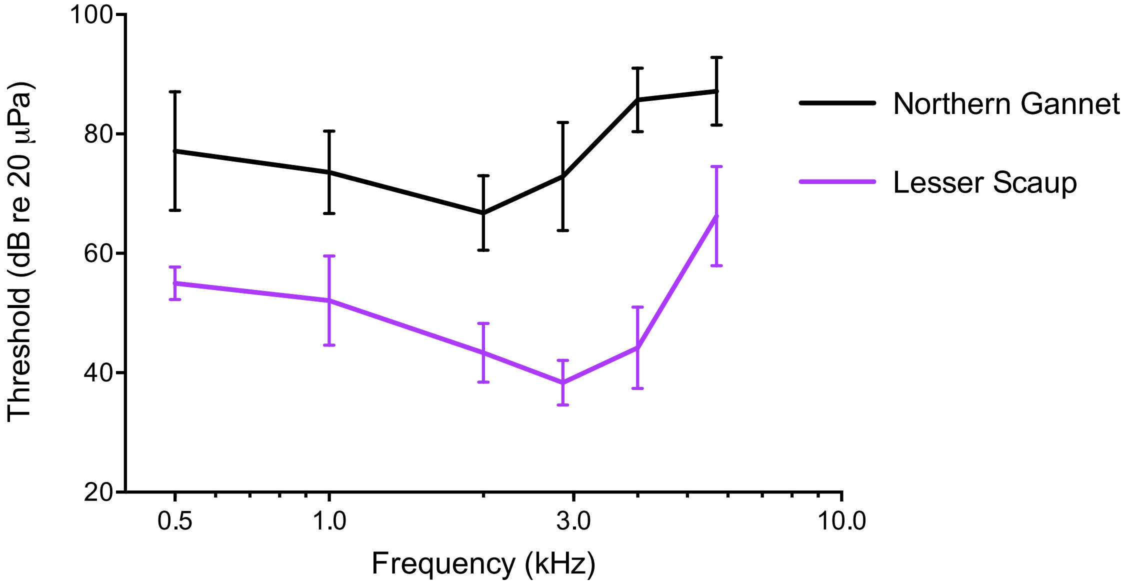

Figure 2.8. Average ABR audiograms from the species with the highest average thresholds (northern gannet) and the lowest average thresholds (lesser scaup). Vertical bars represent +/- one standard deviation.

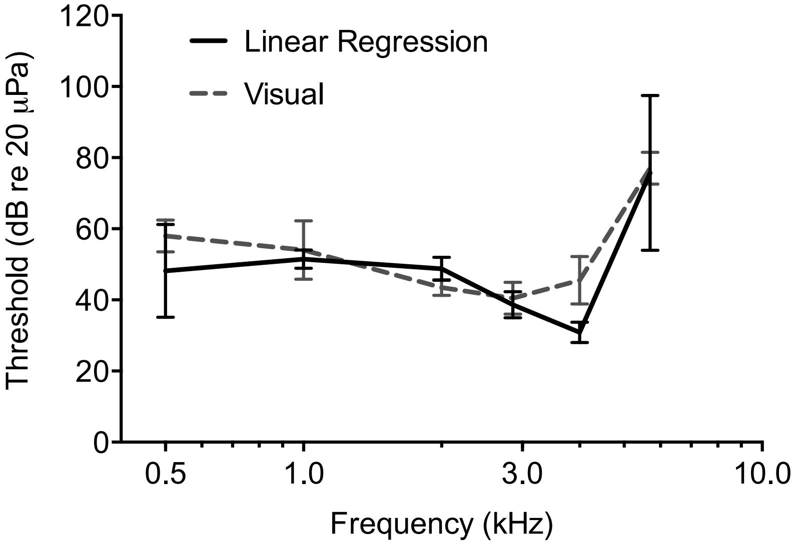

Figure 2.9. Audiograms derived from the two methods of analysis: the visual inspection method, and the linear regression method. Vertical bars represent +/- one standard deviation.

Regression analysis showed there was an inverse relationship between body mass and frequency of best hearing (Figure 2.10, top panel). Nevertheless, the slope of the line is not significantly different from zero and the low r^2 value indicates a high level of variance across points ($y=-0.2288x+2468$, $r^2=0.1704$, $p=0.2357$). There was an apparent linear increase in best frequency of hearing as a function of high-frequency hearing limit, but the trend was not significant at the 5% level (bottom panel of Figure 2.10; $y=0.5987x+3880$, $r^2=0.3600$, $p=0.1544$).

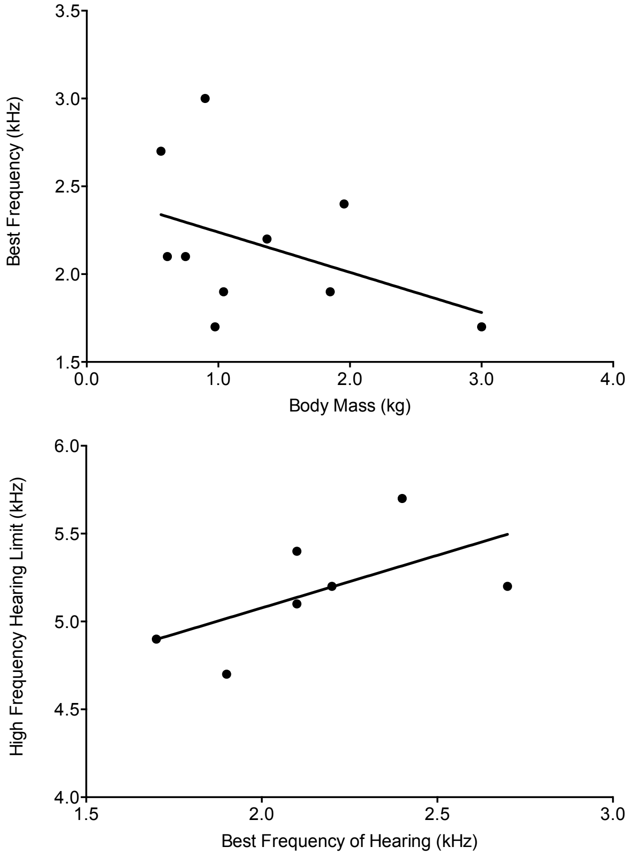

Figure 2.10. Top: No significant difference was found for frequency of best hearing as a function of body mass for ten species based on linear regression analysis ($y=-0.2288x+2468$, $r^2=0.1704$, $p=0.2357$). Bottom: The high frequency limit of hearing as a function of the frequency of best hearing for ten species was not significantly different using linear regression analysis as well ($y=0.5987x+3880$, $r^2=0.3600$, $p=0.1544$).

Anesthesia Analysis

ABR thresholds were compared across two types of anesthesia: the inhalant isoflurane and injectable ketamine/midazolam combination. Four individual lesser scaup were given each treatment (with a two-week washout period between) and their ABRs were measured for each treatment. The anesthesia type did not have a significant effect on thresholds across frequencies ($F_{(1,6)} = 2.02$, $p = 0.1975$).

However, the ketamine/midazolam recovery time was much longer (approximately four hours) than that for isoflurane (less than one hour).

Vocalization Analysis

Ten individual calls from Cornell University's Macaulay Library were measured for eight species (Table 2.2). Samples from two call types for the red-throated loon (the "quark" and the "cry") and from both male and female lesser scaup were analyzed. All other listed species are male calls. All species had average peak frequencies between 1000 and 3000 Hz, with the exception of the common eider at 443 Hz (Table 2.2). Maximum frequency ranged from 1053 Hz (common eider) to 18865 Hz (northern gannet).

Table 2.2. Average peak frequency of vocalizations (frequency at greatest power), maximum frequency of vocalizations, and best hearing frequency for each species.

Species	**Peak Frequency (Hz)**	**Max Frequency (Hz)**	**Best Hearing Frequency (Hz) (n)**
Common eider	443	1053	2400 (10)
Red-throated loon Quark	1528	3439	1900 (6)
Red-throated loon Cry	1983	7857	1900
Black scoter	1714	2012	1900 (3)
Long-tailed duck	1723	12007	2100 (7)
Lesser scaup Male	1779	2850	3000 (only females tested)
Lesser scaup Female	2736	7594	3000 (6)
Northern gannet	2173	18865	1700 (7)
Harlequin duck	2346	3947	2100 (7)

DISCUSSION

The morphology of the ABR waveforms was very similar across all eight duck species tested. These duck ABR waveforms were similar to those exhibited by other birds tested with the ABR technique, such as budgerigars, screech owls, Carolina chickadees (*Poecile carolinensis*), red-winged blackbirds and brown-headed cowbirds (*Molothrus ater*) (Brittan-Powell et al., 2002, 2005; Henry & Lucas, 2010; Gall et al., 2011). The pattern of evoked peaks differed in the two non-duck species (red-throated loons and northern gannets), but still exhibited at least two prominent peaks within 5 ms of stimulus onset.

Other measured characteristics of the ABR responses of all birds measured here also resembled those of other birds and mammals. Specifically, the latency of the first peak increased and the amplitude decreased with decreasing stimulus level, typical of other ABR studies (gerbil, *Meriones unguiculatus,* Burkard & Voigt, 1989; budgerigars, Brittan-Powell et al. 2002; screech owls, Brittan-Powell et al., 2005; Belgian waterslager canaries, *Serinus canaria domestica*, Brittan-Powell et al., 2010; Lohr et al., 2013).

ABR audiograms obtained for each species tested here conformed to the U-shape typical of birds and many other animals. All species tested shared a common region of greatest sensitivity, from 1000 to 3000 Hz, although the audiograms differed significantly across species and frequencies. The significant impact of frequency across the audiogram was expected because of its U-shape, but the species differences are not as easily explained. Species differences in hearing thresholds could be impacted by many factors that are complicated to explore, such as anatomical differences associated with phylogenetic history and/or specific adaptations in skull shape. The thresholds of all duck species tested were more similar to each other than to the two non-duck species tested. The red-throated loon and northern gannet exhibited the highest thresholds, while the lowest thresholds belonged to the ducks, specifically the lesser scaup and ruddy duck.

The northern gannet is the only species of plunge-diver tested here, and has unique adaptations to compensate for hitting the water at speeds up to 100 mph. CT scans done in conjunction with this project at Woods Hole Oceanographic Institution showed extra air spaces in the gannet head and neck to cushion its impact, and these air spaces could affect the ability to observe already small responses from the auditory brainstem (D. Ketten, personal communication, 2012). The tympanic membrane appeared very stiff in the CT scans as compared to the tympanic membrane of the swan, a similar sized bird (D. Ketten, personal communication, 2012). This thickening could be a protective mechanism for plunging and could potentially explain the higher thresholds in gannets that were measured in this study. However, CT scans were only completed with one gannet, so these observations may not be representative of the species.

Gannets may also have the ability to close off its auditory meatus to further streamline the body and avoid the introduction of water into its ear (D. Ketten, personal communication, 2012). Induction of anesthesia, especially when using a mask, can cause diving birds to go into a dive response, when their respiratory rate and heart rate decrease (Machin, 2004). Any other adaptations to diving, including the closing of the meatus to the external environment, could also occur and affect the

ABR. Masks were, however, only used to induce the isoflurane anesthesia. Once a sufficient level of anesthesia was reached, all test birds were intubated and isoflurane delivered through the endotracheal tube. Thus, it is unlikely that diving responses were triggered or present during the actual ABR testing.

The region of peak energy in animal vocalizations is often correlated with the frequency of best sensitivity and/or the bandwidth of the best hearing range (e.g. *bats* – Long & Schnitzler, 1975; Neuweiler et al., 1980; *birds* – Konishi, 1970; Dooling et al., 1971; Dooling & Saunders, 1975; Dooling et al., 2000; *elephants* – Heffner & Heffner, 1982; Payne et al., 1986; *frogs* – Megela-Simmons et al., 1985). Vocalizations of most of the species tested range from frequency-modulated whistles (black scoters), to *purrs* and *whee-oos* (lesser scaup), to loud yodel-like calls (long-tailed duck), to constant chirps (harlequin ducks), to wails (red-throated loons), and to generally silent (white-winged scoter) (Brown & Fredrickson, 1997; Austin et al., 1998; Savard et al., 1998; Robertson & Goudie, 1999; Barr et al., 2000; Goudie et al., 2000; Brua, 2002; Robertson & Savard, 2002; Bordage & Savard, 2011). With the exception of the common eider, the peak frequency (frequency at the greatest intensity) of all species' vocalizations measured here fell between 1000 and 3000 Hz, matching the bandwidth of the most sensitive hearing range. There are some exceptions; the peak frequency of the common eider vocalization (443 Hz) did not match the calculated best hearing sensitivity (2400 Hz). Common eider hearing may be adapted to hear higher-frequency duckling vocalizations, or the mismatch could be a product of environmental conditions or social behavior.

Common eiders and northern gannets are the only colonial nesting species tested in this study. Northern gannets have only six colonies in North America, with the largest, on Bonaventure Island, Quebec, containing more than 73,000 individuals (G. Chapdelaine, unpubl.; Mowbray, 2002). Common eiders commonly nest in densities reaching 100-400 nests/ha (Chapdelaine et al., 1986). In addition, common eiders frequently form dense flocks of up to tens-of-thousands of individuals in the non-breeding season, in response to clumped food resources and possibly heat conservation (Guillemette et al., 1993). Dense, noisy aggregations may favor short-range, more complex auditory cues used for individual recognition amongst thousands of individuals (such as in colonial penguin and auk species – Beecher, 1981; Jouventin, 1982; Jones et al., 1989; Aubin et al., 2000; Lengagne et al., 2000). Like these other colonial seabirds, gannet vocalizations have individually distinctive amplitude envelopes and birds respond preferentially to playbacks of their mate's vocalizations (Nelson, 1978; Mowbray, 2002). The comparatively poor hearing sensitivity of the northern gannet (least sensitive of all species tested) and common eider (least sensitive of all the ducks) may, therefore, reflect the colonial habitat of these species.

The most sensitive hearing of all species tested belonged to the lesser scaup and ruddy duck. In the case of these species, ambient noise levels in the environment may have shaped hearing sensitivity. Of all species tested, ruddy ducks and lesser scaup spend the most time on inland, freshwater environments (Austin et al., 1998; Brua, 2002). Ambient noise levels in stagnant freshwater habitats tend to be consistently lower than in coastal and marine habitats, which are dominated by wind and wave action (Wenz, 1962; Bom, 1969; Urick, 1983; Nystuen, 1986; McConnell et al., 1992; Greene, 1995). There is some evidence that exceptional hearing sensitivity in fishes, such as in the otophysines (carps and minnows, catfishes, characins, knifefishes), may have evolved in quiet freshwater or deep sea habitats (Popper, 1980; Deng et al., 2002, Ladich & Bass, 2003; Amoser & Ladich, 2005). It is possible that sensitive hearing in the ruddy duck and lesser scaup is associated with quiet freshwater habitats, as opposed to the wind- and wave-swept environments occupied by the other birds studied.

Because all birds were tested using the same procedures, species differences in hearing sensitivity were not a result of procedural differences. However, it is possible that species or individuals could vary in their response to the anesthesia. Isoflurane, which was used for all birds in this study, has been shown to elevate thresholds compared to a ketamine/xylazine combination in rats and mice, with a reduction in sensitivity over the duration of anesthesia (Cederholm et al., 2012; Ruebhausen et al., 2012). Prolonged isoflurane anesthesia (8 hours) was also associated with elevated auditory thresholds in the American alligator (Carr et al., 2009).

Isoflurane was chosen, because of its history of effectiveness and safety in waterfowl (Machin, 2004; Carpenter, 2013). While efforts were made to keep the anesthesia level and duration consistent across species (the lowest level possible that would maintain the bird immobile), it is possible that each species could react differently to the anesthesia, or that the waterfowl species could react differently from the non-waterfowl species. In order to determine if thresholds were elevated by the choice of isofluorane, we carried out additional testing on anesthesia type (isoflurane vs. a ketamine/midazolam combination) on a subset of lesser scaup. This was the first comparison of the effects of anesthetic on the ABR in birds, and demonstrated that at least within one species, thresholds did not differ depending on anesthesia type. However, recovery time for the ketamine/midazolam combination was much longer than for isoflurane, illustrating isoflurane's utility for wild birds that have to be released within a short amount of time.

In addition, the season in which the birds were tested, along with the resulting levels of sex hormones, could affect hearing thresholds. Caras et al. (2010) simulated natural breeding or non-breeding conditions by manipulating hormone levels and photoperiod in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), and observed shifts in auditory thresholds, but no significant differences between males and females. During simulated breeding conditions, ABR thresholds were elevated and peak latencies were prolonged compared to non-breeding conditions. While each species was tested within one season (usually within two weeks), the seasons across testing all of the species varied, providing another possible complication for interpreting species differences.

In birds, body mass and basilar papilla lengths are inversely correlated with the most sensitive frequency in a species (Gleich et al. 2005; Corfield et al., 2013). Avian species with a long basilar papilla have the most sensitive frequency of hearing at higher frequencies than do species with a short basilar papilla. The high-frequency limit of hearing is positively correlated with the frequency of best hearing. These relationships were not significant with our data, possibly because the body mass differences across species tested here varied by less than 2500g.

The aquatic bird audiograms obtained, as estimated by the ABR, shared many similarities with other birds tested previously, and also showed considerable variation across species tested. Because there is so little known about the biology and behavior of these species, it would be useful to explore anatomical, behavioral, and evolutionary correlations with these species differences in hearing. Future directions should further investigate the characteristics and use of vocalizations as well as ear anatomy differences across species.

Chapter 3: In-Air Hearing of a Diving Duck, the Lesser Scaup: A Comparison of Psychoacoustics and the Auditory Brainstem Response

INTRODUCTION

The accurate measurement of auditory sensitivity in animals is an important addition to the body of knowledge of a species for which which little information concerning sensory biology is available. Furthermore, non-invasive techniques to measure hearing in animals are valuable tools to learn about species that are not typical laboratory animals. When the opportunity arises to study a species in captivity, it is useful to compare techniques to validate non-invasive methods against standard laboratory techniques. Therefore, the aim of this study was to carry out comparisons of an electrophysiological technique that might be utilized in the field, such as the auditory brainstem response (ABR), with psychoacoustic methods that have been more established as the "gold-standard" of laboratory research (Fay, 1988). For this purpose, the lesser scaup (*Aythya affinis*), a species of diving duck that is not commonly kept in captivity, was used.

Psychoacoustic methods involve training an animal to respond to test stimuli with a particular behavior, such as pressing a lever or pecking a key (e.g. Dooling & Okanoya, 1995; Kastak & Schusterman, 1998; Syzmanksi et al., 1999; Wolski et al., 2003). In contrast, the ABR is an auditory evoked potential, recorded from the scalp, occurring within the first 10 ms following auditory stimulation (Hall, 1992). The recorded series of waves represents synchronized neural discharge during the progressive propagation of auditory neural activity through the ascending auditory pathway (Hall, 1992). The ABR provides a rapid estimate of the shape of the audiogram and range of hearing sensitivity, but thresholds are often 10-15 dB higher than when using behavioral methods (Borg, 1982; Borg & Engström, 1983; Gorga et al., 1988; Brittan-Powell et al., 2002; Wolski et al., 2003; Yuen et al., 2005; Houser & Finneran, 2006; Henry & Lucas, 2008). These elevated thresholds in the ABR can be attributed to differences in stimulus characteristics and measurement techniques between behavioral and electrophysiological methods (Silman & Silverman, 1991; Hall, 1992; Szymanski et al., 1999; Brittan-Powell et al., 2002; Schlundt et al, 2007; Ladich & Fay, 2013; Sisneros et al., 2015). The major advantages of the ABR are that an entire audiogram can often be constructed after one session of less than 60 min, and that there is no animal training involved (it can be used on temporarily-caught wild animals).

Previous studies on lesser scaup and other diving ducks have focused mainly on foraging and reproductive ecology (e.g. Afton & Ankney, 1991; Cutting et al., 2011; Brady et al., 2013; Warren et al., 2014). They are capable of diving to depths of at least 15-18 m, for 2-25 seconds at a time, to forage primarily on mollusks, crustaceans, and aquatic insects (Austin et al., 1998). Both males and females vocalize throughout the year to signal to mates and offspring (Johnsgard, 1965). The lesser scaup is one of the most abundant and widespread species of diving duck in North America, and prefers freshwater, but will winter on brackish bodies of water. Its numbers have been declining in recent years for unknown reasons (Austin et al., 1998). Studies on the sensory biology of this species could elucidate unknown foraging strategies, communication behavior, and habitat selection, and become an important resource in creating an appropriate management strategy if the population continues to decline.

In addition, data on the auditory sensitivity of the lesser scaup will add to current literature on comparative avian hearing. Of the approximately 10,000 extant species of birds, hearing has only been measured in about 50 species (Dooling, 2000, 2003). Approximately half of all birds for which there are hearing data are from the order Passeriformes (perching birds – includes the songbirds), as well as 13 species of owl and several other non-passerine, non-aquatic birds (Dooling, 2000, 2003). There are few data on aquatic birds, such as the lesser scaup. Adaptations for living in an aquatic environment may be related to auditory sensitivity.

The goal of this study was to investigate the auditory sensitivity of the lesser scaup in order to contribute both to the biological knowledge of a species in decline and the overall comparative avian audition literature. Objectives included: 1) use psychoacoustics to obtain absolute auditory thresholds, 2) compare these psychoacoustic results to lesser scaup ABR data from chapter 1, 3) investigate correlations between auditory sensitivity and vocalization parameters, and 4) measure critical ratios.

The critical ratio, or the lowest signal-to-noise ratio at which a tone is detected in broadband masking noise, is calculated as the difference between the masked hearing threshold and the spectral level of the masking noise (Fletcher, 1940; Scharf, 1970). Critical ratios have been used to estimate the frequency selectivity of the auditory system in a variety of animals, including several bird species (Dooling & Saunders, 1975; Lauer et al., 2009; Noirot et al., 2011). Critical ratios also provide a method to verify that ambient noise levels in an experimental setup are not masking absolute thresholds, which is what the data were used for in the present study.

METHODS

The Institutional Animal Care and Use Committees at both the University of Maryland and U.S. Geological Survey Patuxent Wildlife Research Center (where the birds were housed and tested) approved all of the following procedures.

Psychoacoustics

Subjects

Three adult lesser scaup, one male and two female, were used for this study. The three birds were hatched in an incubator in June 2010 and raised together at the U.S. Geological Survey Patuxent Wildlife Research Center's seabird colony. Testing began when the birds were one year old. Thresholds were measured in both quiet and noise (used to calculate critical ratios) for all subjects.

Equipment

Ducks were tested in concrete tanks (2.5 m deep) at the U.S. Geological Survey Patuxent Wildlife Research Center's seabird colony. The testing apparatus consisted of an observation target, report target, automatic mealworm dispenser, and speakers, all at the surface of the water (Figure 3.1). Both targets and mealworm dispenser were made of PVC pipe. Each target was equipped with a light emitting diode (LED) and a pressure-sensitive piezo disk that allowed the computer to record the bird's pecking responses. The observation target was lit with a blue LED, signaling to the duck that they can begin a trial. The response target, used by the duck to indicate the presence of a test signal, was lit with a white LED. The speaker (Frequency response: 90Hz – 20 kHz; Dynex DX-SP211; Richfield, MN) was mounted on the wall of the tank, approximately 30.5 cm in front of the duck when pecking at the observation target. All experimental events were coordinated by a custom computer system (SEABIRD – Sensory Equipment for Animal Behavior and Integrated Research Data; developed by R. Therrien, U.S. Geological Survey Patuxent Wildlife Research Center), powered by a 12V battery. Tones were generated as .wav files using Audacity (opensource) software with a 48k sampling rate. These tones were then stored on an SD card, which was inserted into the SEABIRD hardware. A computer-controlled logarithmic potentiometer attenuated the tones, which were then amplified with a Pyle PLMRMP1A (Brooklyn, NY) before output to speaker. The system was controlled by the user through a touch-screen interface on an Apple iPad (Cupertino, CA).

Calibration of all frequencies and attenuation levels was conducted using a calibrated Earthworks M30 microphone (Milford, NH) at the location where the bird's ear would be, connected to the iPad with an Alesis iO ProAudio Dock (Cumberland, RI). The iPad was running SignalScope Pro software (Faber Acoustical; Santaquin, UT), which has an FFT analyzer function to perform real-time spectral analysis in 1 Hz spectral levels. The system was calibrated with a CEM SC-05 sound level calibrator (Shenzhen, China). In addition, a daily calibration was performed, during which a 60 dB tone was played across all frequencies, measured by a BK Precision 732A sound level meter (Yorba Linda, CA), which fed back into the SEABIRD hardware to provide voltage adjustments. Daily variation in decibel levels before calibration was +/- 3 dB.

Ambient noise in the experimental set up was also measured using the Earthworks M30 microphone connected to the iPad. Ambient spectral levels were visualized using SignalScope Pro on the iPad.

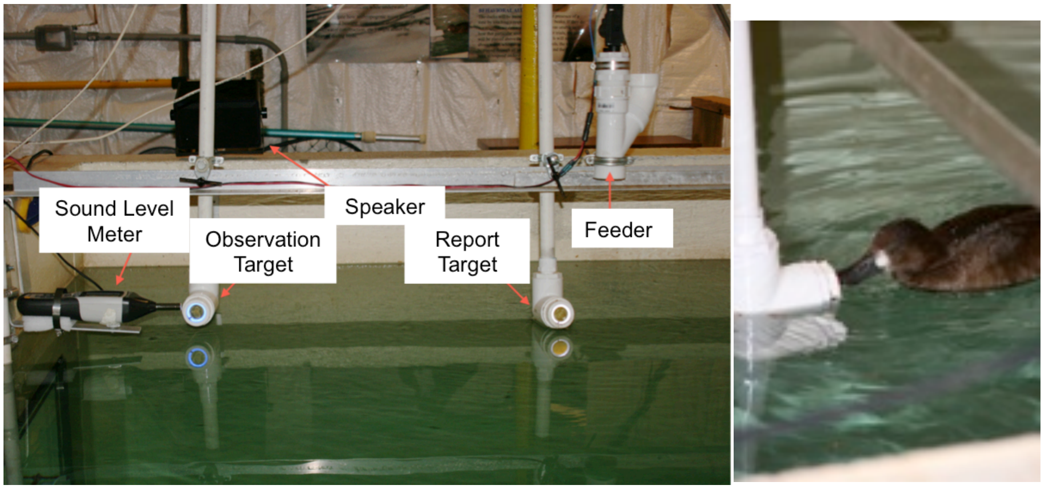

Figure 3.1. Left: Experimental set-up for in-air hearing test, including two targets and a mealworm feeder, all made of PVC. Right: Lesser scaup in training session pecking at a target.

Training and Testing Procedures

An individual duck was transferred to the tank from its outdoor pen before trials began. Ducks were trained using operant conditioning procedures on a go/no-go task. Each duck was trained until reliably performing above 90% accuracy, at which time testing commenced.

At the beginning of a trial, both the observation and target were illuminated. To begin a trial, a duck pecked the lit observation target. Each time the bird pecked the observation target, the computer generated a random number from 1-10. When the peck random number was from 7-10, the trial would go to completion, either with the playback of a tone or a sham trial, and the target lights would shut off. If the peck to the observation target generated a number from 1-6, the lights would stay on and the trial would continue, waiting for further pecks. If a tone was played, the duck had to peck the report target within four seconds. If the duck pecked the report target correctly (hit), a variable number of mealworms were delivered as a reward and the target lights shut off for a random interval of 15s +/- 5s. If a tone was played and the duck failed to report (miss), no mealworm was delivered and the trial ended with the target lights shut off. If no tone was delivered (sham trial), the duck was to refrain from hitting the report target until the target lights shut off (correct rejection). The bird's rate of response during sham trials was used to calculate the false alarm rate. If the duck pecked the report target in the absence of a tone (false alarm), the target lights shut off and the duck received a 10-second "punishment" period when the lights in the building were shut off.

At the beginning of each testing session, the bird was trained with 5-10 warm-up trials, during which the bird was presented with a pre-selected stimulus level well above threshold. This regular training allowed the bird a daily adjustment period to the task. Birds were tested once per day, and were allowed to test until they lost interest, signified by five minutes passing without pecking the observation target. Sessions of less than twenty trials were discarded. To measure critical ratios, all testing procedures were the same, except with the addition of the broadband noise described below.

Stimuli

Stimuli consisted of two pure tone 1000-ms sinusoidal pulses separated by 500 ms, with a 250-ms rise time, a 500-ms steady state peak, and a 250-ms fall time. Hearing sensitivity was measured for frequencies of 0.5, 1.0, 2.0, 2.86, 4.0, 5.7, and 8.0 kHz. The signal frequency was held constant for each session. The order of frequencies tested was random, but was the same across birds. Each block consisted of ten trials – seven intensity levels and three sham trials. The seven intensity levels were pre-selected in steps of 10 dB. These levels were adjusted until one stimulus intensity was below threshold, the next was near threshold, and the remaining five were above threshold. During each block, the seven intensity levels and three sham trials were presented in random order.

For critical ratio trials, masking noise was played continuously throughout the session. White noise was also generated using Audacity software, digitally filtered to be flat (+/- 5dB) between 0.5 and 8.0 kHz (also using Audacity), and integrated into the hardware system with a Behringer MicroMix MX400 (Bothell, WA). The noise was played at two levels (20 dB/Hz and 30 dB/Hz or 55 dB and 65 dB overall), for each frequency tested. Spectral levels were calibrated using the same microphone/iPad system described above for tone calibration. Masked thresholds were measured at 1.0 kHz and 2.86 kHz.

Threshold Estimation

The ten-trial blocks were added together across consecutive days until the bird completed 100 trials. Threshold was estimated after each of these 100-trial sets. The birds were tested repeatedly at each frequency until threshold values across these 100-

trial sets showed no further improvement (the threshold was within +/- 1/3 of the step size for three sets of 100 consecutive trials). The final threshold estimate was then defined as the mean threshold estimate from the last 200 trials, at an SPL corresponding to a 50% correct detection rate. False alarm rate was also calculated for each set of 100 trials. One-hundred-trial sets with false alarm rates higher than 15% were discarded.

Threshold was also defined according to Signal Detection Theory, to account for each bird's individual false alarm rate. The discriminability index d' was calculated at all frequencies for each bird with its false alarm rate and a 50% hit rate. In addition, new hit rates were calculated using each bird's false alarm rate and a d' of 1.0, 1.5 and 2.0. The SPL corresponding to these new hit rates at each frequency were then calculated for a new measurement of threshold. Critical ratios were calculated by subtracting the spectrum level of the noise from the masked threshold.

Vocalization Analysis

Adult male and female lesser scaup vocalizations were obtained from Cornell University's Macaulay Library collection. Lesser scaup duckling vocalizations were recorded at Patuxent Wildlife Research Center (Sound Devices 702 portable recorder, Reedsburg, WI). Spectrographic analysis of minimum, maximum, and peak frequency (the frequency of the greatest power) was performed using Raven Lite 1.0 (Cornell Lab of Ornithology; Ithaca, NY). These measurements were then compared to the most sensitive hearing frequency and high-frequency limit of hearing. Hearing sensitivity was calculated in 100 Hz steps for the range of frequencies tested by fitting the raw audiogram data points to a third-order polynomial (Gleich et al., 2005). The frequency of best hearing was then defined as the lowest 100 Hz point on this curve. The high-frequency limit of hearing was defined as the point on this curve where threshold rises >30 dB above the lowest threshold.

Auditory Brainstem Response

Subjects

The subjects for this portion of the study were of the same species, but different individuals, as those tested in the psychoacoustic portion. Although it would be valuable to test the same individuals using both methods, the risk of putting the trained psychoacoustics birds under anesthesia to measure the ABR outweighed the benefits to the study. ABR subjects were six adult lesser scaup, raised from eggs at U.S. Geological Survey Patuxent Wildlife Research Center.

Experimental Procedures

Experimental procedures were described in detail in chapter 1. All birds were sedated with isoflurane (2-4%; the lowest possible percentage was used to prevent movement in the bird) prior to electrode placement. The bird was positioned so that the speaker was 20 cm from the bird's right ear. Electrodes were placed subdermally high on the bird's forehead (active), directly behind the right ear canal (the ear ipsilateral to the speaker, reference), and behind the canal of the ear contralateral to stimulation (ground).

Subjects were presented with tone burst stimuli 5 ms in duration with a 1 ms rise/fall and a 20 ms interstimulus interval, at frequencies between 0.5 and 5.7 kHz. The stimuli were presented in trains consisting of nine single frequency tone bursts that increased successively in intensity and were presented at a rate of 4/s (see chapter 1 and Brittan-Powell et al., 2002, 2005, 2010).

The stimulus presentation and ABR acquisition were coordinated using Tucker-Davis Technologies (Gainesville, FL, USA) hardware and OpenABR software (Dr. Edward Smith, University of Maryland). Stimulus intensities were calibrated in the free field by placing a 1/4 microphone (Earthworks M30-Calibrated) at the approximate position of the animal's ear (20 cm from the speaker).

Latency and Amplitude:

The amplitude and latency of the first peak was measured for all frequencies and intensities tested. The latency was corrected for the delay between the speaker and the bird's ear (0.59 ms). The amplitude of the first peak was determined by subtracting the average voltage from the section of the waveform before the response began from the peak (peak to baseline measurement).

Threshold Estimation

Threshold was defined using two methods: visual detection and linear regression. In the visual detection technique, the first 10 ms of all ABR waveforms were examined visually by a trained observer for a response. Threshold was defined as the level one half step below the lowest stimulus level at which a response could be visually detected on the trace (see Brittan-Powell & Dooling, 2004; Brittan-Powell et al., 2005, 2010; Lohr et al., 2013). Thresholds were also estimated using linear regression, as described in chapter 1: The amplitude of the first positive peak was obtained across all frequency and stimulus levels and an amplitude-intensity function was generated. Threshold was defined as the 0 μV crossing of a line produced with linear regression.

RESULTS

Psychoacoustics

Audiogram

Using psychoacoustics, three lesser scaup (identified by colored leg bands as Pink, Yellow, and Blue) were tested at frequencies from 0.5 Hz to 8.0 kHz. Less than 10% of sessions for each bird were discarded because of a false alarm rate higher than 15% (0% for Pink bird, 3% for Yellow bird, and 8.8% for Blue bird). Psychometric functions for all three birds at 1.0 kHz are shown in Figure 3.2. In this example, at least one stimulus level was well below threshold, one level was slightly above threshold, and four stimulus levels were well above threshold, and responded to close to 100% of the time. Each symbol on the figure represents an average percent correct for the last twenty trials tested at 1.0 kHz. Threshold corresponded to a hit rate of 50%, which was equal to 27.5 dB re 20 μPa for Pink bird, 23.7 dB re 20 μPa for Yellow bird, and 24.5 dB re 20 μPa for Blue bird.

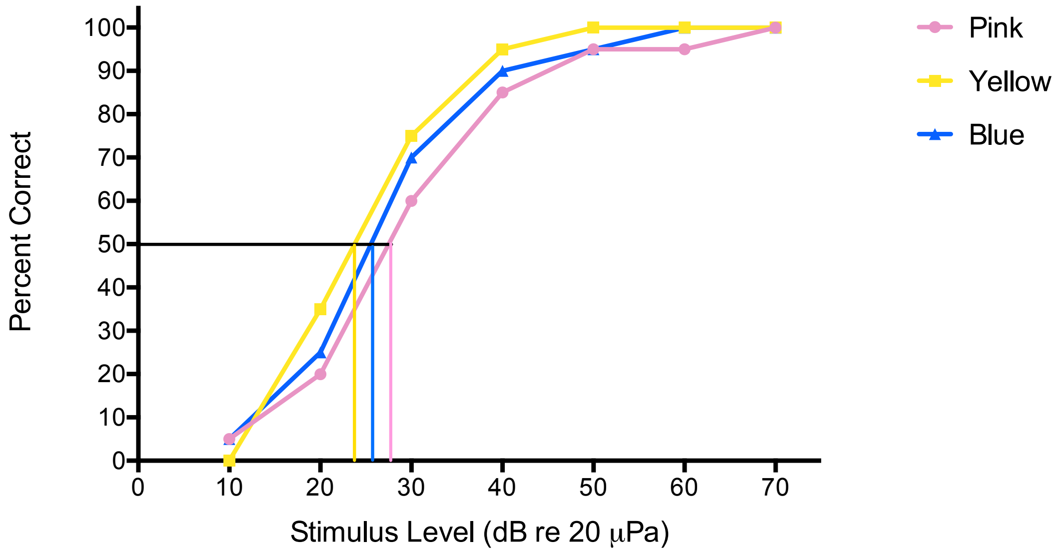

Figure 3.2. Psychometric functions for three lesser scaup (identified by colored leg bands) at 1.0 kHz. Each symbol represents twenty trials.

All three birds tested displayed best sensitivity at 2.86 kHz, with an average threshold of 14.4 dB, corresponding to a hit rate of 50%. The high-frequency roll-off above 4 kHz was much steeper than the low frequency roll-off. Audiograms for all birds are shown in Figure 3.3. Figure 3.4 depicts the average scaup audiogram along with the spectrum level of the ambient background noise in the building.

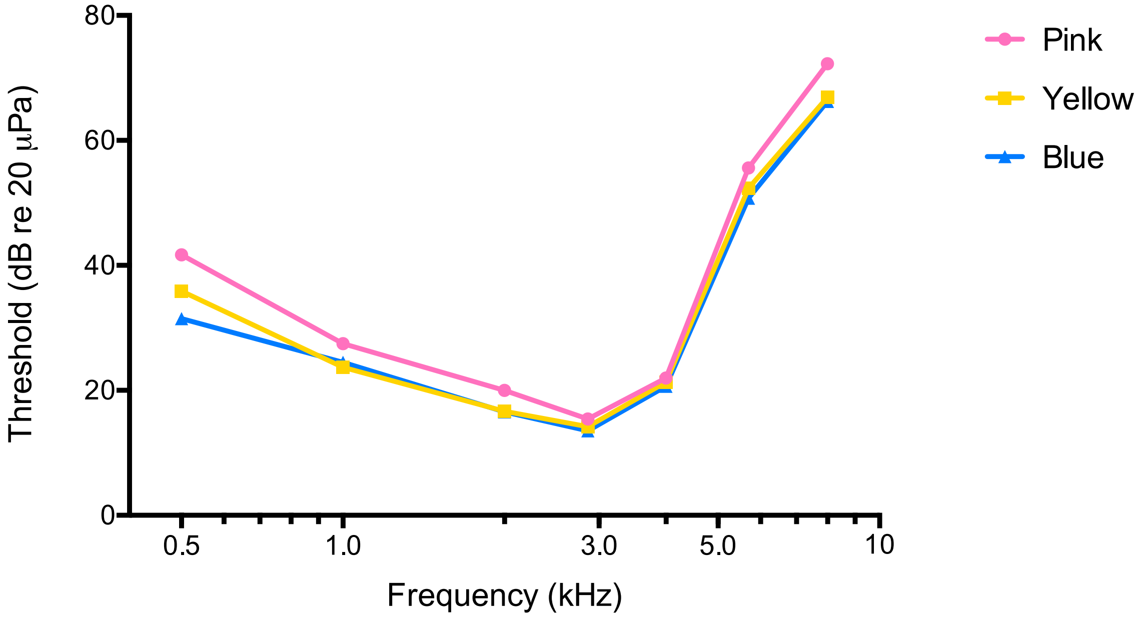

Figure 3.3. Audiograms for all three birds tested, corresponding to a hit rate of 50%.

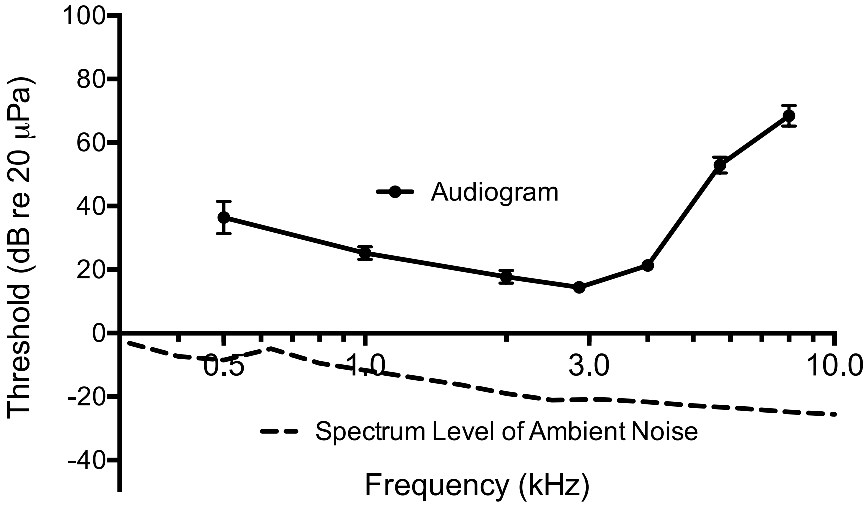

Figure 3.4. Average scaup audiogram with background noise (plotted in spectrum levels) in the dive tank building. Spectrum levels were measured using SignalScope Pro software on an iPad. Vertical lines represent +/- 1 standard deviation.

Signal Detection Theory was used to account for each bird’s individual response bias. Across all frequencies, the 50% performance level corresponded to a d' of 1.91 for Pink bird, 1.48 for Yellow bird, and 1.25 for Blue bird. Threshold at each frequency was also calculated for each bird performing at three d' levels: 1.0, 1.5, and 2.0. To do this, a new hit rate at each frequency was calculated to correspond to the bird's false alarm rate and each level of d'. This new hit rate was then used to define a new threshold level. Figure 3.5 displays the average audiogram (using a hit rate of 50%) from all three birds along with the new audiograms estimated using d' of 1.0, 1.5, and 2.0. From this figure, it is apparent that defining threshold using a 50% hit rate corresponds most closely to a d' of 1.5.

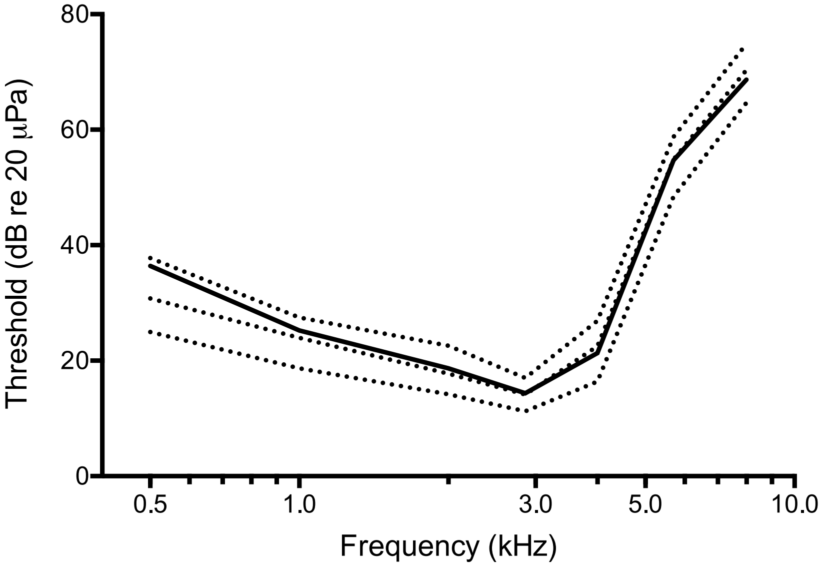

Figure 3.5. Audiograms from three lesser scaup. The solid line represents thresholds defined using a hit rate of 50%, and the dotted lines represent hit rates calculated using a d' of 1.0, 1.5, and 2.0.

Critical Ratios

Critical ratios were measured at 1.0 kHz and 2.86 kHz. The frequency of test tone, spectrum level of the masking noise, the average masked threshold, and the average critical ratio are reported in Table 3.1. Masked thresholds increased in proportion to noise spectrum level, while critical ratios remained relatively constant.

Table 3.1. Frequency of test tone, masking noise level, masked threshold and calculated critical ratio averaged across all lesser scaup.

Frequency (Hz)	Masking Noise Level (dB re 20 µPa/Hz)	Masked Threshold (dB re 20 µPa)	Critical Ratio (dB) + [SD]
1000	20	41.5	21.5 [1.83]
1000	30	54.5	24.5 [2.0]
2860	20	41.9	21.9 [1.66]
2860	30	52.9	22.9 [2.12]

Vocalization Analysis

Recorded vocalizations from lesser scaup ducklings and adult males and females were analyzed for several measurements (Table 3.2). Because of limitations on sample sizes, males and females were lumped together for hearing measurements, and therefore the frequency of best hearing and high-frequency limit of hearing were calculated across both sexes. Hearing tests were not conducted on ducklings. Adult female vocalizations were more broadband in nature, spanning a wider range of frequencies, and with a higher peak frequency, than the adult male vocalizations. The peak frequency of the duckling vocalizations was higher than both the male and female vocalizations.

Table 3.2. The average minimum frequency, maximum frequency, and peak frequency (frequency at greatest power) of male, female and duckling vocalizations, along with the calculated frequency of best hearing, and high-frequency limit of hearing. These two measurements were only calculated for adult lesser scaup, and males and females were not separated.

	Min Freq (Hz)	**Max Freq (Hz)**	**Peak (Dominant) (Hz)**	**Best Hearing (Hz)**	**High-Freq Limit* (Hz)**
Male	709	2850	1779	2400	5300
Female	391	7594	2736	2400	5300
Duckling	2441	5724	4061		

Auditory Brainstem Response

The typical lesser scaup auditory brainstem response displayed 2-3 prominent peaks within 4-5 ms after the stimulus reached the bird's ear canal (Figure 3.6). As with other birds tested, ABR latencies increased and amplitudes decreased as stimulus level decreased. These data are those first presented in chapter 1.

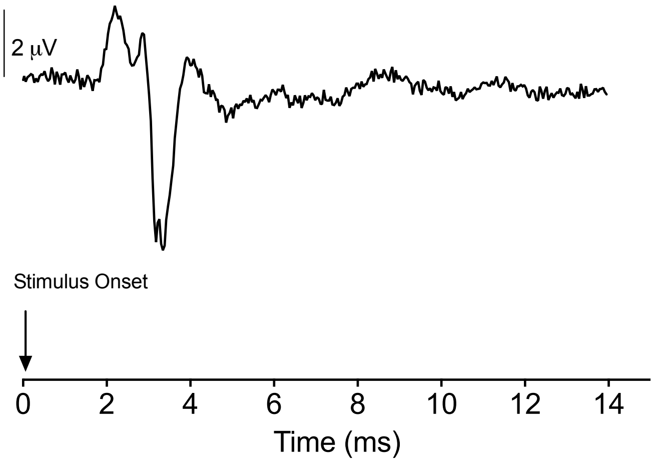

Figure 3.6. A typical auditory brainstem response from a lesser scaup with a 2.86 Hz, 90 dB (measured at the ear) tone pip as the stimulus. This figure represents an average of 600 responses.

The ABR audiograms estimated with the visual inspection method and the linear regression method were both U-shaped. Sensitivity peaked between 1.0-3.0 kHz, with a steep high-frequency roll-off after 4.0 kHz. Threshold estimates did not differ between the visual inspection method and the linear regression method across frequencies (F(1,8)=2.5, *p* = 0.15; two-way repeated measures ANOVA). Figure 3.7 compares the visual inspection ABR audiogram to the psychoacoustic audiogram. Both methods produced U-shaped audiograms with similar regions of greatest sensitivity (from 1-4 kHz). ABR thresholds were higher than psychoacoustic thresholds at all frequencies tested (ABRs were not measured at 8 kHz). Differences ranged from 11.3 dB at 5.7 kHz to 26.8 dB at 1.0 kHz.

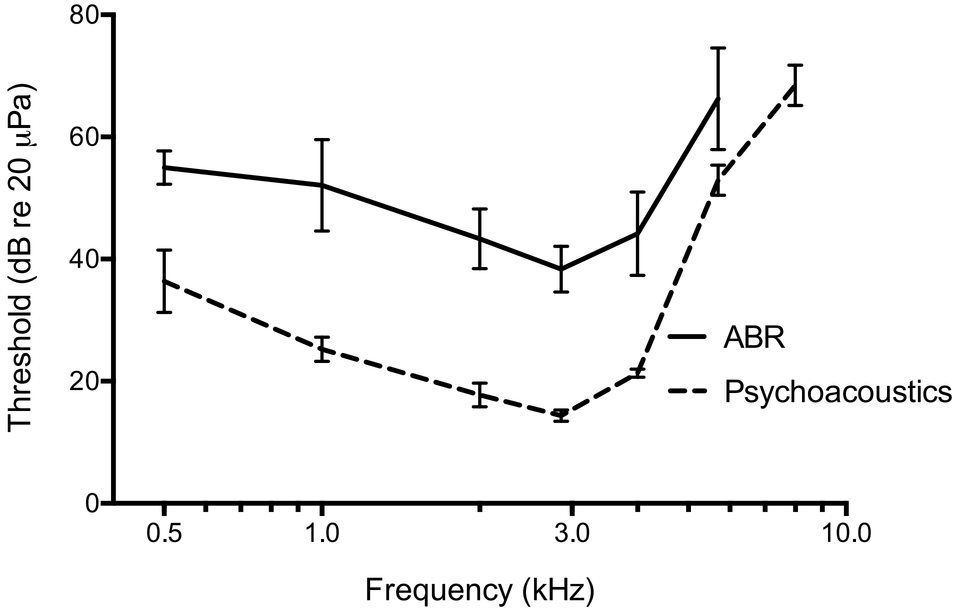

Figure 3.7. A comparison of audiograms using the auditory brainstem response and psychoacoustics. The ABR was not measured at 8 kHz. Vertical bars represent +/- 1 standard deviation.

DISCUSSION

Psychoacoustics

The average lesser scaup behavioral audiogram was U-shaped, with sensitivity peaking at 2.0-3.0 kHz, and an absolute threshold of approximately 14 dB. Existing data from over 50 species of birds tested to date reveal a typical avian pattern of greatest sensitivity between 2000 and 5000 Hz (Dooling et al., 2000, chapter 1). The lesser scaup displayed a low frequency roll off of approximately 10 dB per octave below 1.0 kHz, and a much steeper high frequency roll off above 4.0 kHz (approximately 50 dB per octave). Average avian absolute thresholds in the region of peak sensitivity approach 0 dB, with a loss of sensitivity below 1.0 kHz of about 20 dB/octave and a loss of sensitivity above 4.0 kHz of about 60 dB/octave (Dooling et al., 2000). The only behavioral audiogram available for another non-diving duck species, the mallard duck, also follows this pattern (Trainer, 1946).

The application of signal detection theory provides some information on how the subject's sensitivity interacts with bias in detection tasks (Green & Swets, 1966). Although subjects' criterion can be manipulated by changing aspects of reinforcement (amount/timing of food reward, presence/absence of punishment, etc.), subjects will adopt criteria with some amount of individual variation (Herrnstein, 1961; Green & Swets, 1966; Lattal, 1979; Mandell, 1981; Nevin, 1981). Some of this individual variation can be accounted for using false alarm rates and different d' levels. For the lesser scaup in this study, thresholds were estimated using a hit rate of 50%, as well as using d' levels of 1.0, 1.5, and 2.0. When these threshold estimates were compared, the 50% hit rate tracked most closely to a d' of 1.5, which is similar to other birds performing detection tasks (Dooling & Saunders, 1975; Dooling & Okanoya, 1995).

In this study, critical ratio measurements allowed us to verify that the estimated thresholds in quiet actually approached absolute levels. Critical ratios for the lesser scaup were estimated at two frequencies, one of which was the frequency of most sensitive hearing (2.86 kHz). The average critical ratio at 2.86 kHz was 22.5 dB, very similar to that reported for the budgerigar (19.9 dB) and canary (*Serinus canaria domestica*, ~20 dB) (Dooling & Saunders, 1975; Lauer et al., 2009). Background noise in the tanks at Patuxent Wildlife Research Center was quieter than one critical ratio below the threshold at 2.86 kHz, suggesting that the absolute thresholds reported in this study were not masked by ambient noise.

Like other birds, hearing in lesser scaup aligns with species-specific vocalizations. The vocalization peak power in several avian species, including the downy woodpecker (*Picoides pubescens*), hairy woodpecker (*Picoides villosus*), and budgerigar, corresponds well to the most sensitive hearing range (Dooling & Saunders, 1975; Lohr et al., 2013). Henry and Lucas (2008, 2010) suggested that in several songbird species (Carolina chickadees, *Poecile carolinensis*, tufted titmice, *Baoelophus bicolor*, house sparrows, *Passer domesticus*, and white-breasted nuthatches, *Sitta carolinensis*), the high-frequency limit of sensitive hearing may have co-evolved with the maximum frequency of vocalizations. Male and female vocalizations of lesser scaup are dimorphic in nature. Males are generally quieter, but emit a whirring, kazoo-like “whew,” or “whee-ooo,” often referred to as a coughing call, during courtship (Johnsgard, 1965). Female scaup are louder and more frequently vocal. They produce a noisy “purrr” during courtship, in the presence of predators, and also to inform mates and ducklings when they are returning to the nest (Johnsgard, 1965). Both sexes primarily vocalize while sitting on water, and rarely while flying (Austin et al., 1998). The average peak frequency of both the male (1.779 kHz) and the female (2.736 kHz) vocalizations align with the region of greatest sensitivity on the audiogram, and the calculated frequency of best sensitivity (2.4 kHz). The range of frequencies in lesser scaup duckling vocalizations (2.441-5.724 kHz) also aligns well with both the frequency of best sensitivity as well as the high-frequency limit of sensitive hearing (5.3 kHz). Thus, it would appear that the lesser scaup has hearing abilities that correspond well to both the adult and duckling vocalizations, leading to improved chances for cooperative foraging and predator detection, courtship, and nest success.

Comparison of ABR and Psychoacoustic Audiograms

Both ABR and psychoacoustic measures yielded audiograms with similar U-shapes and regions of greatest sensitivity. However, ABR thresholds were higher than psychoacoustic thresholds at all frequencies. This difference was least at the highest frequency tested using both methods (5.7 kHz) and greatest at 1.0 kHz, where the ABR threshold was 26.8 dB higher. This difference could be attributed to a variety of factors, including stimulus characteristics (brief ABR stimuli vs. longer psychoacoustics stimuli), physiological state of the subjects (anesthetized for the ABR and awake for psychoacoustics), individual differences in hearing abilities (different subjects were used for each method), and the nature of the two methods. Disparities between psychoacoustics and the ABR have been documented in many animal groups, but differences between the two methods appear to be greatest in avian species, including screech owls (*Megascops asio*), budgerigars, tufted titmice, house sparrows, white-breasted nuthatches, and finches (Wooley & Rubel, 1999; Brittan-Powell et al., 2002, 2005; Henry & Lucas, 2008). The only other data available for a duck species, the mallard, also displays this disparity (Trainer, 1946; Dmitrieva & Gottlieb, 1992). This difference may be a consequence of the ability to detect responses in mammals vs. birds, since mammals on average have a greater absolute number of auditory nerve fibers than birds, and a greater proportion of fibers activated at threshold (Brittan-Powell et al., 2002).

Conclusions

Used in conjunction, psychoacoustics and the ABR were complementary methods to test hearing in lesser scaup. Audiograms produced maintained the same shape and region of greatest sensitivity, regardless of method used. The ABR is therefore a valuable tool to provide a rapid (under an hour) estimate of hearing, especially with animals that cannot be trained. For most animal species, the total number of individuals tested is such a small fraction of the population that there is no real consensus on individual variation of hearing and how this variation may affect current assumptions about a species-specific audiogram. The ABR should continue to be used to increase this sample size and better characterize hearing abilities across individuals and species, especially in animals that are not typically kept in captivity. In birds, psychoacoustics may remain the “gold standard” for measuring hearing, and should continue to be used when possible to verify results obtained with other methods.

Chapter 4: Underwater Hearing in a Diving Bird

INTRODUCTION

Underwater hearing abilities have been examined in species of dolphins, whales, seals, sea lions, fishes, turtles, frogs, and even some invertebrates (Terhune & Ronald, 1975; Thomas et al., 1988; Christensen-Dalsgaard et al, 1990; Römer & Tautz, 1991; Budelmann, 1992; Kastak, 1996; Christensen-Dalsgaard et al., 2012; see Fay, 1988 for a compilation of results from many species). However, there are currently no measurements of underwater hearing by any diving bird. Diving birds may be subject to the same potential impacts of man-made noise sources as many of these other aquatic organisms, but it is impossible to comprehensively examine possible effects of increased noise levels on hearing by an aquatic animal without first investigating its auditory sensitivity and how it processes sound.

Man-made noise sources may be impulsive, such as explosions, pile driving, and seismic sources, or continuous, such as commercial and recreational shipping noise. All have the potential to mask communication, mask valuable aspects of the soundscape, deter animals from preferred foraging or breeding habitat, disrupt predator-prey interactions, and/or cause temporary or permanent hearing impairment (e.g. Finneran & Schlundt, 2004; Nowacek et al., 2004; Finneran et al., 2010; Finneran & Schlundt, 2010; Parks et al., 2012; Richardson et al., 2012; Tougaard et al., 2012; Tyack & Janik, 2013). Extreme impulse noise can cause physiological damage, such as rupture of the tympanic membrane, fracture of the middle ear ossicles, or non-auditory tissue injury (e.g. Keevin & Hempen, 1997; Hamernik et al., 2002; Davis et al., 2009; Carlson, 2012; Casper et al., 2012; Le Prell, 2012).

Most studies on the effects of man-made noise sources on birds concern vehicular noise. Urban songbirds alter their communication signals to avoid the loudest frequencies of traffic noise (Slabbekoorn & Ripmeester, 2008; Slabbekoorn, 2013). Traffic noise, as well as noise associated with oil and gas extraction, is also associated with a decline in the diversity and density of songbirds (Rheindt, 2003; Reijnen & Foppen, 2006; Bayne et al., 2008; Francis et al., 2009). None of these studies, however, involve aquatic birds, and without measurement of underwater hearing sensitivity in birds, it is impossible to predict susceptibility of aquatic birds to underwater noise.

While often complicated to implement, measurements of hearing abilities of animals underwater have been carried out in a number of species. A variety of measurements, including both behavioral and auditory evoked potential audiograms, have been employed to test hearing in marine mammals and fishes (Gerstein et al., 1999; Wolski et al., 2003; Houser & Finneran, 2006; Mooney, 2009; Ladich & Fay, 2013). Such measurements are lacking in birds, although in-air audiograms exist for many avian species (Dooling et al., 2000).

Of the 29 extant orders of birds, eight orders contain species that dive to some extent while foraging: Anseriformes (ducks, geese, and swans), Charadriiformes (gulls and allies), Gaviiformes (loons), Podocipediformes (grebes), Procellariiformes (albatrosses, petrels, and allies), Sphenisciformes (penguins), and Phaethontiformes (tropicbirds). Time spent underwater ranges from minimal plunges in gulls and waders to extensive diving in penguins and puffins, with emperor penguin dives documented to depths of over 500m (Meir et al., 2008). At the shallow end of the diving continuum, some duck species of the family Anatidae can dive to depths of tens of meters for up to a few minutes (Roberston et al., 2002). The long-tailed duck (*Clangula hyemalis*) is the deepest diver of all ducks, reaching at least 60 m in depth to search for crustaceans, fishes, and mollusks (Roberston et al., 2002).

Long-tailed ducks are potentially impacted by man-made noise sources during their long migration away from remote Arctic and subarctic breeding grounds. The Atlantic flyway population of long-tailed ducks winter along the coast from Labrador south to North Carolina, as well as Hudson Bay and the Great Lakes (Silverman et al., 2012). They spend the majority of their time in coastal marine waters near major population centers along the Atlantic coast where they are potentially exposed to a variety of in-air and underwater noise sources, including construction from coastal development, commercial shipping, and recreational vessels. In addition, their flight paths and wintering areas overlap with potential wind-energy development sites (Sea Duck Joint Venture, 2012). Offshore wind farms are associated both with impulsive noise sources, such as pile driving during construction, as well as continuous maintenance noise (Maxon, 2000; Henriksen, 2001).

Given their coastal proximity and exposure to a variety of man-made noise sources, long-tailed ducks provide a useful model for the first investigation of underwater hearing in a diving bird. In addition, access to a captive breeding population and diving facility provided the opportunity to raise and train ducklings to participate in a long-term behavioral study. Therefore, the objective of this study was to measure the in-air and underwater auditory sensitivity of long-tailed ducks, using psychoacoustic methods. Sensitivity of the long-tailed ducks was compared in-air and underwater, and underwater sensitivity was compared to that of marine mammals and fish.

METHODS

Subjects

Two long-tailed ducks, one male (with a pink identification leg band) and one female (with a yellow leg band), were used in the study. Sex of birds was identified through plumage patterns and cloacal inspection for phallus protrusion. Both birds were raised from eggs at the Patuxent Wildlife Research Center in July of 2012. Testing began when the birds were approximately one year old. The male bird died unexpectedly in the fall of 2013 so the remainder of the testing was conducted with the female bird. Therefore, the male bird was only able to complete about half of the underwater testing, and no in-air testing. The female participated in both in-air and underwater testing.

In-Air Testing Procedure

All in-air and underwater experimental events were coordinated by a custom computer system (SEABIRD – Sensory Equipment for Animal Behavior and Integrated Research Data; developed by R. Therrien, U.S. Geological Survey Patuxent Wildlife Research Center), described in more detail in chapter 3. In-air equipment, procedures, stimuli, and calibration were described in detail in chapter 3, and summarized here. The ducks were tested in rectangular concrete tanks (2.4 m length x 1.8 m width x 2.4 m depth). The testing apparatus consisted of an observation target, report target, automatic mealworm dispenser, and speakers (Figure 4.1). During in-air testing, the observation target was at the surface of the water and the speaker was mounted on the wall of the tank, approximately 30.5 cm in front of the duck when pecking at the observation target.

In-air stimuli consisted of two pure tone 1000-ms sinusoidal pulses separated by 500 ms, with a 250-ms rise time, a 500-ms steady state peak, and a 250-ms fall time. Hearing sensitivity in-air was measured for frequencies of 0.5, 1, 2, and 2.86, 4.0, and 5.7 kHz.

Ambient noise in the experimental set up was also measured using the Earthworks M30 microphone connected to the iPad. Ambient spectral levels were visualized using SignalScope Pro on the iPad.

Underwater Testing Procedure

During underwater trials, the observation target was lowered approximately 33 cm below the surface of the water, and the speaker (University Sound UW-30; Electro-Voice, Burnsville, MN) was mounted on a bracket from the front wall of the tank, 30.5 cm in front of the duck when pecking the observation target (Figure 4.1). The report target and mealworm dispenser always remained at the water surface.

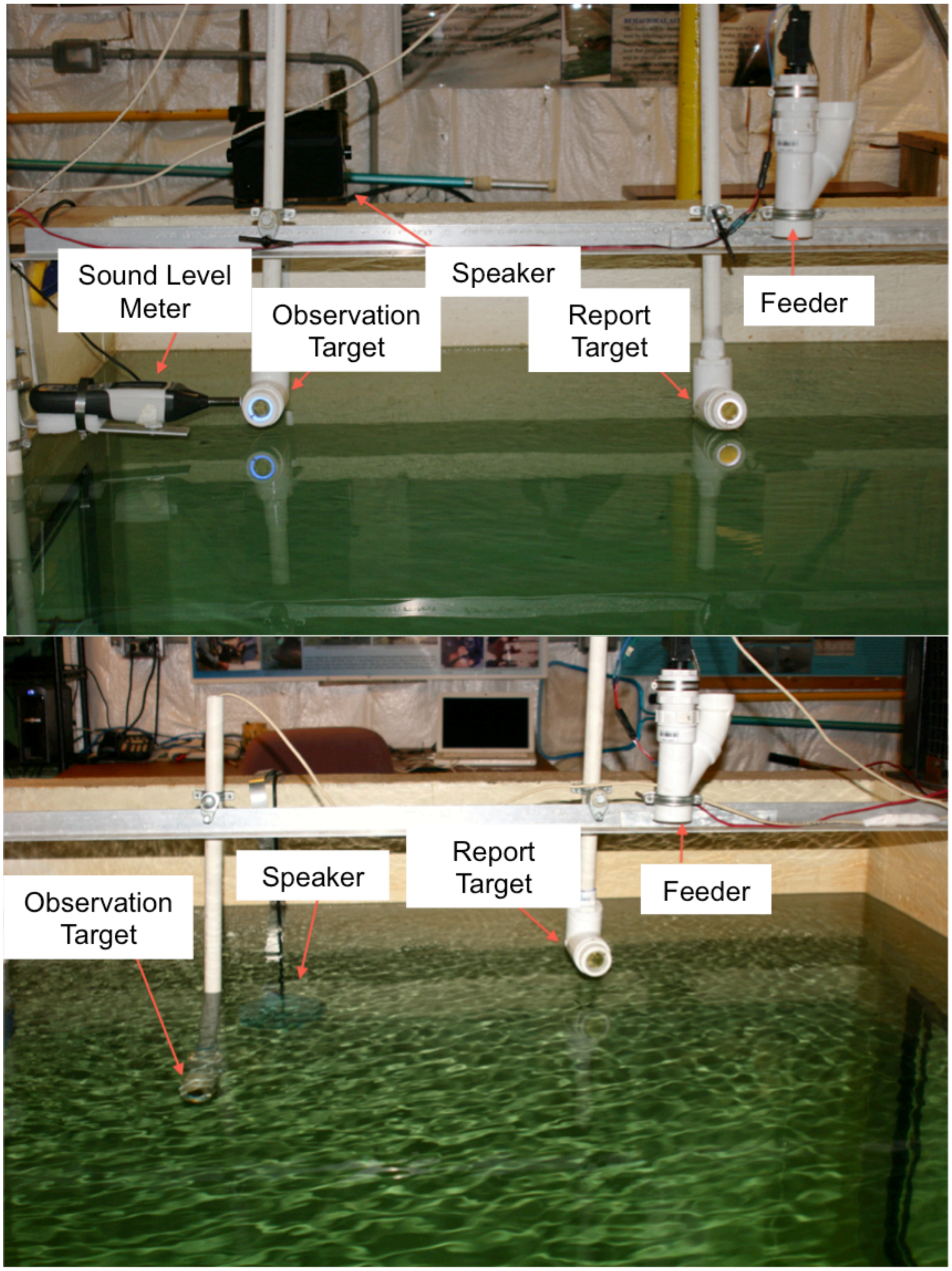

Figure 4.1. In-air (top) and underwater (bottom) equipment set-up. Both set-ups included a speaker mounted from the front wall of the tank, 30.5 cm in front of the observation target. The stimulus was played from the speaker, and the bird reported the presence of a stimulus by pecking the report target. After a stimulus was correctly reported, the bird was rewarded with mealworms that dropped out of the feeder.

In-air and underwater training and testing procedures were similar. The ducks were trained on the go/no-go task until reliably performing with above 90% accuracy. When a trial began, both targets were illuminated. The duck then had to peck the observation target in air or underwater until either a tone was played or the target lights turned off. If a tone was played, the duck then had four seconds to peck the report target (hit) before the target lights turned off and the trial ended. If no tone was played (sham trial) the trial also ended (correct rejection). If a tone was played and the duck failed to respond (miss), the trial also ended with no reward. If no tone was played and the duck pecked the report target (false alarm), the trial ended and the lights in the building were extinguished for 10 seconds as a punishment. The bird's rate of response during sham trials was used to calculate false alarm rate.

Given short diving times and the requirement that the duck station at the observation target prior to stimulus onset, the underwater stimulus was a single 1000-ms sinusoidal pulse, and rise fall times of x ms. Hearing sensitivity was measured for frequencies of 0.5, 1, 2, and 2.86 kHz. The method of constant stimuli was used, and the signal amplitude varied randomly among seven pre-selected levels at each frequency tested (Table 4.1), ranging from 82 to 142 dB re 1 μPa. Some levels were repeated more than once per block at 0.5 and 1.0 kHz, but only the first incidence of each stimulus level per block was used for analysis. Higher levels at these frequencies were repeated to avoid having too many stimuli well-below threshold, and to avoid having additional levels above 142 dB re 1 μPa (because of speaker distortion and risk of hearing damage).

Table 4.1. Frequencies and stimulus levels used in testing.

Frequency (kHz)	**Level 1 (dB re 1 µPa)**	**Level 2 (dB re 1 µPa)**	**Level 3 (dB re 1 µPa)**	**Level 4 (dB re 1 µPa)**	**Level 5 (dB re 1 µPa)**	**Level 6 (dB re 1 µPa)**	**Level 7 (dB re 1 µPa)**
0.5	102	112	122	132	132	142	142
1.0	92	102	112	122	132	142	142
2.0	82	92	102	112	122	132	142
2.86	82	92	102	112	122	132	142

To calibrate underwater stimuli, a receiving hydrophone with preamplifier (Teledyne-Reson 4032, Slangerup, Denmark; sensitivity = -170 dB re 1 V/µPa) was mounted in the position at which the bird's ear would be located while pecking at the observation target. Calibration stimuli were the same stimuli used in testing, except repeated ten times. During playback of the calibration tones, the peak output voltage of the hydrophone was measured on an oscilloscope (Rigol DS1052E, Beijing, China), and this voltage measurement was used to calculate the equivalent decibel level. The potentiometer attenuation values were then adjusted until the decibel level matched the desired level. Every stimulus level was calibrated separately for the frequency that was being tested. This calibration was conducted weekly.

Further measurements of calibrated stimuli with a signal analyzer (Hewlett Packard 35665A, Palo Alto, CA) revealed variation in stimulus generation of up to +/- 5 dB at some stimulus levels. This variation was not observed in the in-air set up, and the source of this variation was unidentified. Because of this, while the stimulus values were nominally in steps of 10 dB from 82 to 142 dB re 1 µPa, a few steps, as measured by the signal analyzer, were up to 15 dB. This affected the method of analysis for the data, described below.

Using the nominal 10 dB steps, the birds were tested repeatedly at each frequency until threshold values showed no further improvement (the threshold, corresponding to the 50% correct detection level, was within 4 dB for 300 consecutive trials). Sessions with false alarm rates higher than 15% were discarded. However, because of the measured variation in stimulus values, a final threshold was not defined. Instead, the variation was taken into account by grouping the stimuli at each frequency into "low," "medium," and "high" stimulus levels (Table 4.2). For example, the nominal 82 and 92 dB re 1 μPa stimulus levels were grouped in a "low" stimulus level, and +/- 5 dB was added on either side to describe the variation that the birds actually heard during testing.

Table 4.2. Stimulus groupings for analysis purposes. Stimulus were grouped into three levels (low, medium, and high), and ranges for each of these groups were calculated taking into account a possible +/- 5 dB variation at each level.

Stimulus Grouping	**Nominal Levels Included**	**Range with +/- 5 dB Variation**
Low	82, 92 dB re 1 μPa	77 – 97 dB re 1 μPa
Medium	102, 112 dB re 1 μPa	97 – 117 dB re 1 μPa
High	122, 132, 142 dB re 1 μPa	117 – 147 dB re 1 μPa

After these groupings were defined, the percentage correct for each of the groupings was calculated using the final 200 trials at each frequency. Instead of defining threshold at a particular decibel level, threshold was defined as falling between two groupings (i.e. between "medium" and "high" at 1.0 kHz).

RESULTS

In-Air

In-air auditory sensitivity of one long-tailed duck (the female) was measured at frequencies between 0.5 and 5.7 kHz. The percentage of sessions discarded because of a false alarm rate of 15% or higher was 3.8%. Figure 4.2 displays the in-air audiogram of the long-tailed duck and to validate this curve average in-air audiogram from three lesser scaup (*Aythya affinis*), another duck species tested using the same procedures and equipment (chapter 2), are also included. Each symbol on the figure represents an average threshold for the last twenty trials tested at that frequency and decibel level. The long-tailed duck's greatest sensitivity was at 2.0 kHz, with a threshold of 10.2 dB re 20 μPa, corresponding to a hit rate of 50%. The high frequency roll-off above 3.0 kHz was much steeper than the low frequency roll-off. Across all frequencies, the 50% performance level corresponded to a d' of 1.57.

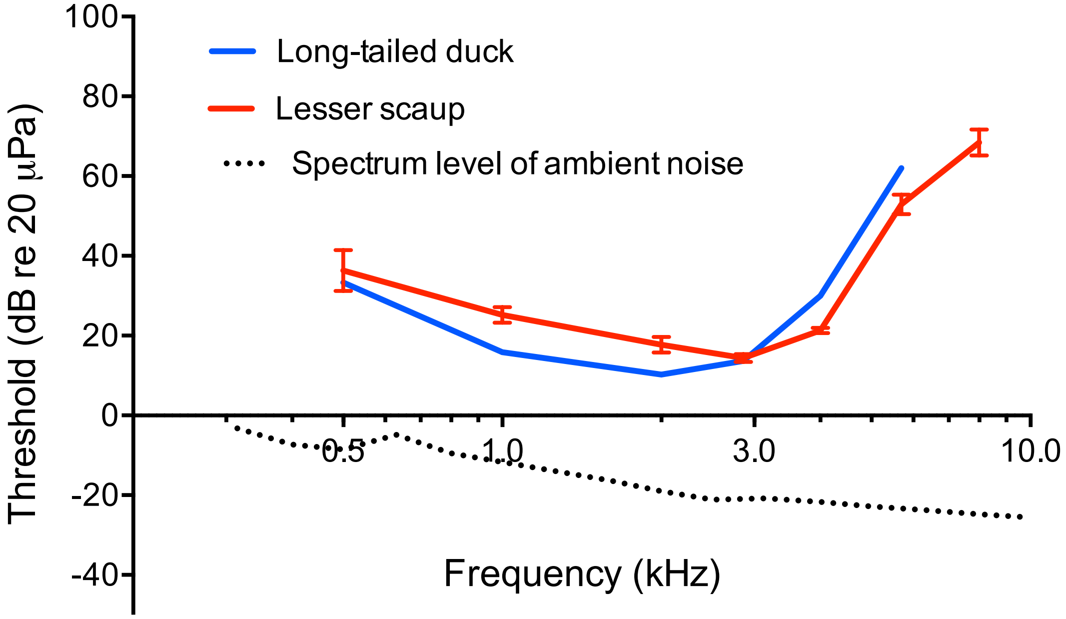

Figure 4.2. The long-tailed duck in-air audiogram, plotted with the average in-air lesser scaup audiogram from chapter 3. The lesser scaup and long-tailed duck were tested in the same experimental set-up. Also plotted is in-air background noise (plotted in spectrum levels) in the dive tank building. Spectrum levels were measured using SignalScope Pro software on an iPad.

Underwater

Two long-tailed ducks participated in underwater sensitivity testing. One bird (Pink) completed testing at 0.5, 2.0, and 2.86 kHz, and began testing at 1.0 kHz before he died. The other bird (Yellow) completed testing at 2.0 and 2.86 kHz. Less than 10% of sessions for each bird were discarded because of a false alarm rate higher than 15% (5% for Pink bird and 0% for Yellow bird).

Percentages correct at each stimulus grouping (low, medium, and high) and for each frequency are reported in Figure 4.3 and Table 4.3. Because the lowest stimulus level at 0.5 kHz fell above the “low” grouping, there are only results for the “medium” and “high” grouping at that frequency. While all other percentages represent the last two hundred trials, the threshold at 1.0 kHz only represents the first 100 trials of testing at that frequency, before the bird's health declined.

Table 4.3. Percentage correct at each stimulus grouping and frequency for each bird. Pink bird was tested at all four frequencies, and Yellow bird only at 2.0 and 2.86 kHz. Pink bird's results at 1.0 kHz only represent the first 100 trials at that frequency, after which the bird's health declined.

Frequency (kHz)	**Low (77-97 dB re 1 µPa)**	**Medium (97-117 dB re 1 µPa)**	**High (117-147 dB re 1 µPa)**
0.5 Pink	N/A	25.0%	81.7%
1.0 Pink	5.0%	57.5%	95.0%
2.0 Pink	12.5%	80.0%	96.7%
2.0 Yellow	15.0%	70.0%	96.7%
2.86 Pink	12.5%	72.5%	98.3%
2.86 Yellow	5.0%	60.0%	93.3%

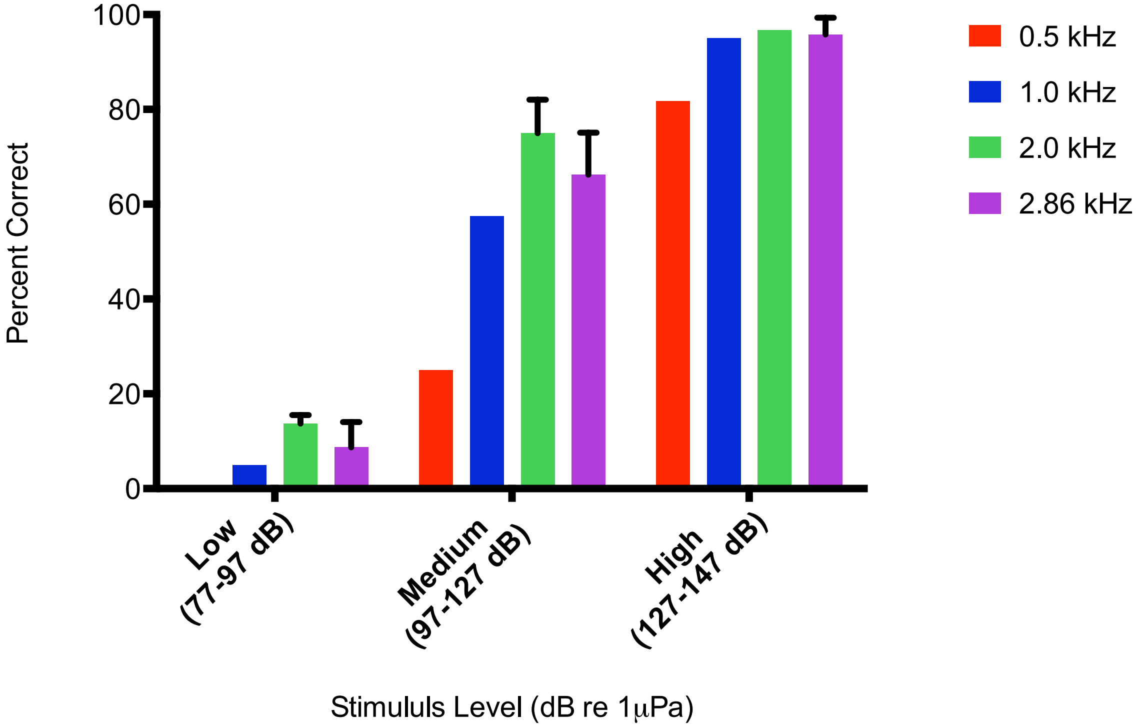

Figure 4.3. Percentage correct at each of the three stimulus groupings. Because the lowest stimulus level at 0.5 kHz fell above the "low" grouping, there are only results for the "medium" and "high" grouping at that frequency. Vertical bars represent standard deviations at frequencies with results from two birds (2.0 and 2.86 kHz).

Threshold was defined only as falling between two stimulus groupings (the two groups that bracketed 50% correct detection). These threshold ranges are plotted as boxes alongside underwater audiograms from several marine mammal and fish species in Figures 4.4 and 4.5. The figures only include behavioral thresholds (no auditory evoked potentials) to provide consistency in measurement methods. Figure 4.4 displays the entire audiogram for all species listed, and Figure 4.5 displays only frequencies up to 10 kHz, to provide more detail in the long-tailed duck's likely underwater hearing range.

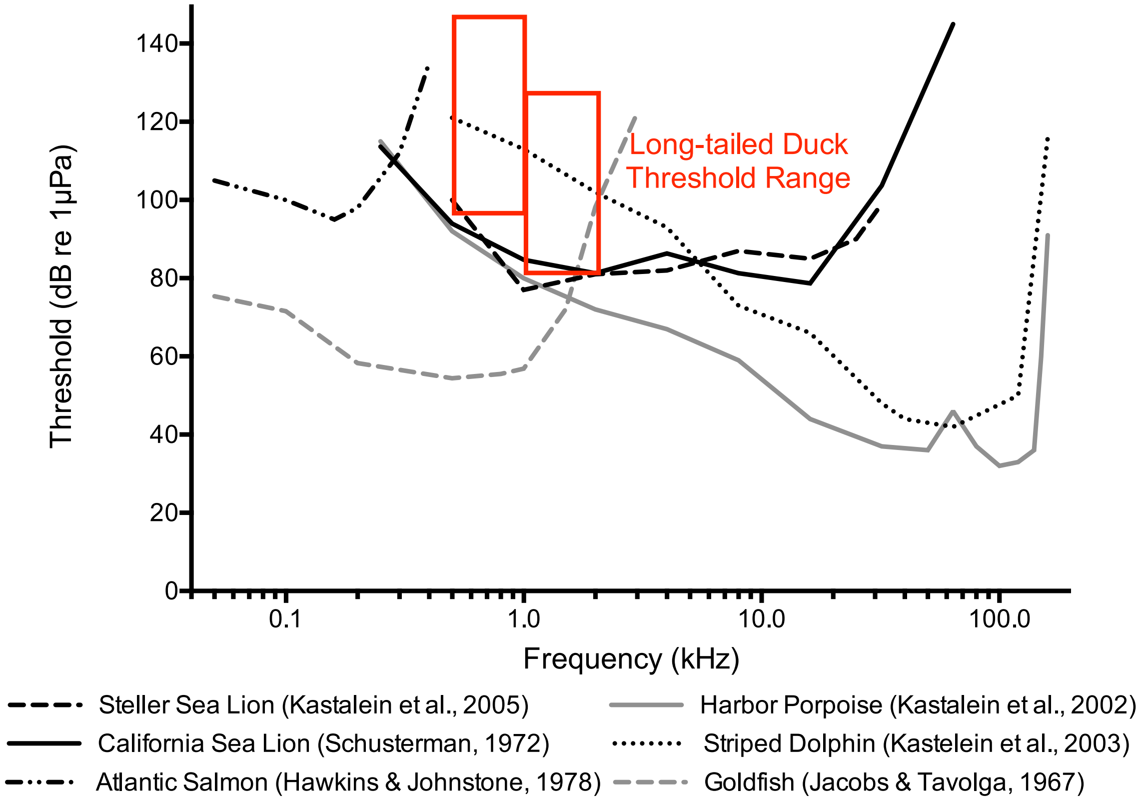

Figure 4.4. The range of the long-tailed duck's underwater thresholds at all frequencies tested, along with underwater behavioral audiograms from the Steller sea lion (*Eumetopias jubatus*), California sea lion (*Zalophus californianus*), harbor porpoise (*Phocoena phocoena)*, goldfish *Carassius auratus*), Atlantic salmon (*Salmo salar*), and striped dolphin (*Stenella coeruleoalba)*.

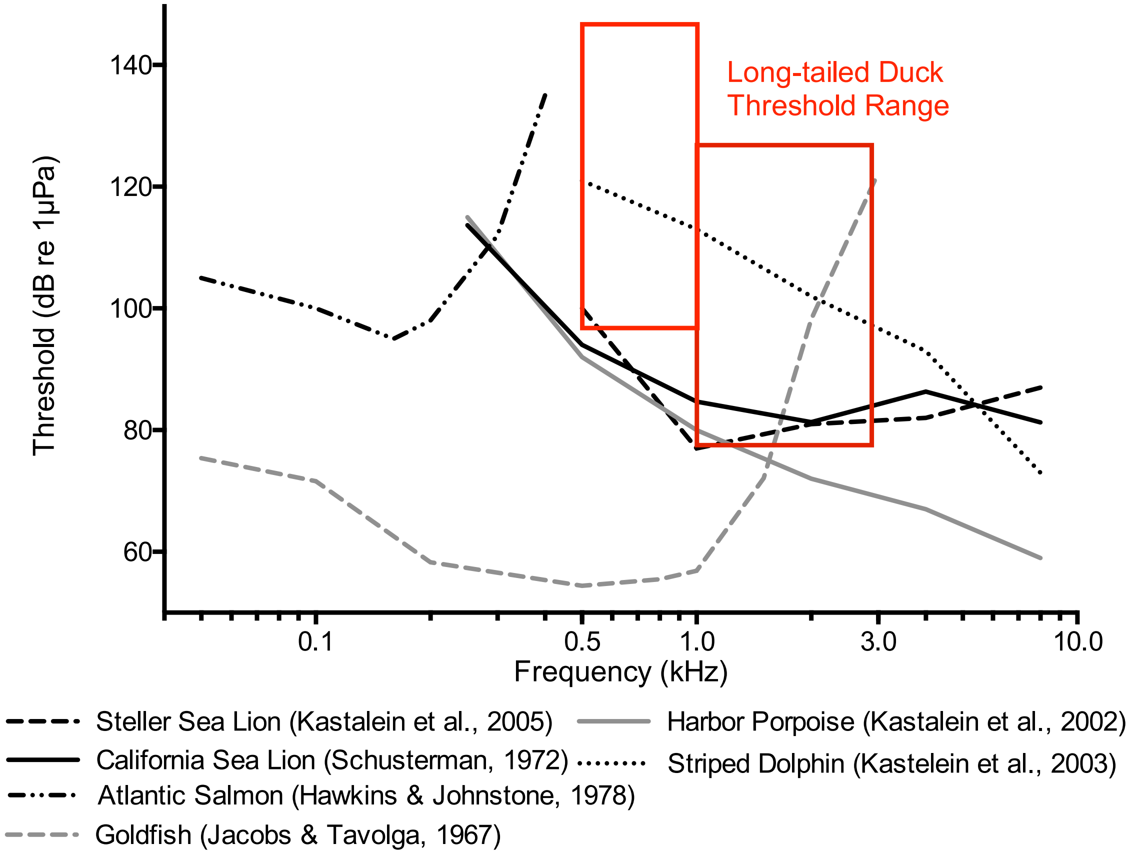

Figure 4.5. The range of the long-tailed duck's underwater thresholds at all frequencies tested, along with underwater behavioral audiograms, displayed up to 10 kHz, from the Steller sea lion, California sea lion, harbor porpoise, goldfish, Atlantic salmon, and striped dolphin.

DISCUSSION

In air, the long-tailed duck heard much like the lesser scaup, another member of the family Anatidae, tested in chapter 2. Sensitivity was best at 2.0 – 3.0 kHz, with a steeper high-frequency roll-off than at lower frequencies. Both the lesser scaup and the long-tailed duck had audiograms that coincided with the typical U-shaped avian audiogram, with most birds having best sensitivity between 2.0 and 5.0 kHz (Dooling et al., 2000).

Signal detection theory was used to provide information on how the subject's sensitivity interacts with bias in this task (Green & Swets, 1966). All thresholds in this study were estimated using a hit rate of 50%, which corresponded roughly to a d' of 1.5 across all frequencies, both in the air and underwater. This d' value is similar to other birds performing detection tasks (Dooling & Saunders, 1975; Dooling & Okanoya, 1995; chapter 2).

This study represents the first measurements of underwater auditory sensitivity in any bird. Underwater sound production and reception of marine mammals and fish have been investigated for much of the last century, while diving birds have garnered little attention in this respect. The long-tailed ducks in this study were tested underwater at frequencies from 0.5 kHz to 2.86 kHz. Although exact threshold points could not be determined because of variation in the stimulus generation system, threshold for 0.5 kHz falls in the range of 97 – 147 dB re 1 μPa, and thresholds for 1.0, 2.0 and 2.86 kHz fall in the range of 77 – 127 dB re 1 μPa. Many marine mammals tested, and especially the odontocetes, have audiograms with much greater absolute sensitivity (35-55 dB re 1 μPa, Johnson, 1967; Thomas et al., 1988; Nachtigall et al., 1995; Sauerland & Denhardt, 1998; Kastalein et al., 2002; Kastalein et al., 2003).

Odontocetes, however, are fully aquatic, and therefore are not adapted to hearing in air. Amphibious animals, like diving birds, pinnipeds, frogs, and some turtles, may be adapted to hear in both air and water. Maximum underwater sensitivity is relatively consistent across pinniped species, but in-air hearing varies greatly across species, without any consistent correlation with amount of time spent in the water, diving ability, or sound production (Schusterman, 1972; Kastalein et al., 2005; Au & Hastings, 2008; Reichmuth et al., 2013). Frog species tested, however, do show a correlation between underwater hearing sensitivity and degree of aquatic adaptation (Lombard et al., 1981; Christensen-Dalsgaard et al., 1990).

The ability to dive spans many orders of birds, with a correspondingly large range in diving behavior and physiology. Since the long-tailed duck is currently the only bird with underwater auditory sensitivity measurements, it is difficult to predict how underwater hearing could correlate with adaptations for an aquatic lifestyle in birds. The only suggestion that diving birds may vocalize underwater came from recordings of a single macaroni penguin at the Moscow Zoo (Markov, 1977), but no subsequent studies have documented underwater sound production in any bird.

In the absence of underwater communication, diving birds may use hearing for auditory scene analysis. Long-tailed ducks and related diving duck species may use gradations in the soundscape (the collection of sounds that emanate from landscapes – Pijanowski et al., 2011) to locate suitable foraging areas. Seaducks are often observed foraging around oyster reefs, which provide habitat for many species of mollusks, crustaceans, and fishes, all important foraging items (Perry et al., 2007). Several studies have shown that reefs form a distinct soundscape, with generally higher sound levels compared to adjacent soft-bottom habitats, generated by the population of invertebrates and fish that inhabit the reef (Simpson et al., 2008; Lillis et al., 2013). Listening for these soundscape characteristics would be an efficient method to find suitable foraging habitat for diving birds.

The mechanisms underlying underwater hearing in diving birds are difficult to predict. Birds may hear through the traditional outer-middle-inner ear route that they utilize in the air. However, an air-filled middle ear could create an impedance mismatch underwater and a loss of sensitivity, and it is possible that diving birds may hear through bone conduction, as suggested for pinniped underwater hearing (Repenning, 1972; Ramprashad, 1975; Hemilä et al., 2006), and much like human hearing underwater (Brandt & Hollien, 1969; Hollien & Brandt, 1969; Repenning, 1972). Adaptations for diving, such as the closing of the external auditory meatus or venous tissue in the middle ear (such as in the king penguin – Sade, 2008), could also impact the sensitivity and mechanism of underwater hearing. This venous tissue causes the middle ear to fill with blood to compensate for increasing pressure on the tympanic membrane as the animal dives (Repenning, 1972; Stenfors et al., 2001). It is possible that when this tissue is flooded with blood, acoustic conductance could occur through the traditional tympanic route (Møhl, 1968; Repenning, 1972; Moore & Schusterman, 1987; Terhune, 1989).

Underwater sounds have two components, particle motion and sound pressure. Particle motion is generated by the hydrodynamic flow near the acoustic source (Rogers & Cox, 1988; Radford et al., 2012). Particle motion dominates in the area close to the acoustic source, termed the near-field and defined as $\lambda/2\pi$, and the far-field is defined as the area outside the near-field, where sound pressure dominates (Bergeijk, 1967; Rogers & Cox, 1988; Higgs et al., 2006; Montgomery et al., 2006; Maruska et al., 2007; Radford et al., 2012). It is possible, although unlikely, that some of the tones presented in this study could have been perceived through acoustic particle motion and not sound pressure. Although auditory thresholds in this study were not measured in terms of particle motion, the frequencies tested in these experiments were typically higher than those that would be used to test particle motion sensitivity. Because the near-field at the frequencies tested in this study is very small, it can be assumed that the bird is positioned in the far-field while listening to the stimuli, and that the particle motion contribution is likely not detected.

While results of this study cannot be used to generate an exact audiogram for long-tailed ducks underwater, the results show that this species can hear underwater at the frequencies tested and thus may be potentially impacted by man-made noise sources underwater. If diving birds do indeed use soundscape cues to assist with foraging (e.g. to find oyster reefs), it is possible that man-made noise sources could mask these cues. If a bird is diving in the proximity of an intense impulsive event (like pile driving during construction of wind farms), it could potentially be susceptible to physiological injury like other animals exposed to impulsive noise (e.g. Keevin & Hempen, 1997; Hamernik et al., 2002; Davis et al., 2009; Carlson, 2012; Casper et al., 2012; Le Prell, 2012).

Conclusions

Underwater hearing was measured for the first time in a diving bird. The birds reliably responded to high intensity stimuli (above approximately 120 dB re 1 μPa). These first measurements highlight the need for further investigation into underwater hearing in diving birds. The large differences in diving behavior across bird orders suggest that it is crucial to extend this study to other diving bird species in order to understand how birds perceive sound underwater. Further behavioral, physiological and anatomical investigations should be conducted to elucidate the sensitivity and mechanism of hearing in diving birds. In respect to the impacts of anthropogenic noise sources such as construction, seismic surveys, military sonars, etc. on aquatic animals, diving birds should receive attention comparable to that paid to other aquatic animals such as marine mammals and fish.

Chapter 5: General Discussion

Hearing has been examined in only a small fraction of extant bird species, and in only two aquatic bird species, the black-footed penguin (Wever et al., 1969) and mallard duck (Trainer, 1946). Aquatic bird species are scattered throughout the avian phylogeny, and their adaptations for an aquatic lifestyle may impact hearing sensitivity. This research was an exploration of aquatic bird hearing across species and in-air and underwater.

In the first set of experiments, the in-air auditory sensitivity was compared across two species of diving duck, six species of seaduck, one species of loon, and one gannet species. Auditory brainstem response (ABR) methodology showed a U-shaped audiogram, a region of greatest sensitivity (1.0 – 3.0 kHz), and a waveform morphology similar to other bird species tested in previous studies (Brittan-Powell et al. 2002, 2005; Henry & Lucas, 2010; Gall et al., 2011). Among the aquatic bird species tested, the waveform morphologies of the duck species were more similar to each other than to the two non-duck species. The lesser scaup had the lowest ABR thresholds, and the northern gannet had the highest. Variation between duck and non-duck species in-air hearing abilities may be indicative of divergent life history traits, morphology, and behavioral characteristics.

Psychoacoustic methods were then used to explore the in-air hearing of one species of diving duck, the lesser scaup, more in depth. Psychoacoustics produced a U-shaped audiogram, with sensitivity peaking at 2.0 – 3.0 kHz, and an absolute threshold of approximately 14 dB re 20 μPa. The ABR yielded an audiogram with a similar shape and region of maximum sensitivity, but with thresholds up to 26.8 dB higher. This difference can be attributed to either the methodological differences inherent in the two types of tests (stimulus differences, analysis differences, etc.), the physiological state of the subjects (awake vs. anesthetized), or individual differences in hearing abilities.

Psychoacoustic methods were then used to measure the in-air and underwater hearing sensitivity of the long-tailed duck. The in-air audiogram resembled that of the lesser scaup tested in the previous chapter, except with peak sensitivity at 2.0 kHz, instead of 2.86 kHz. Because of the difficulties inherent in measuring underwater acoustic stimuli, it was not possible to construct an exact audiogram underwater, but birds were trained to reliably respond to underwater acoustic stimuli. At all frequencies tested (from 0.5 kHz to 2.86 kHz), birds responded to high-intensity stimuli (approximately 120 dB re 1 μPa and greater) greater than 80% of the time. Thresholds were defined in ranges, with 0.5 kHz falling between 97 and 147 dB re 1 μPa, and frequencies of 1.0, 2.0 and 2.86 kHz falling between 77 and 127 dB re 1 μPa.

The aim of this dissertation was to explore the in-air and underwater auditory sensitivity of aquatic birds to add to the literature on avian hearing. Furthermore, very little biological research has been devoted to the group of birds examined in this dissertation. Many of these species are widely distributed, spend a great deal of time far offshore, and migrate long distances, making them particularly difficult to study. Basic biological information for most species is lacking, and data suggest that many of these species are declining drastically (North American Waterfowl Management Plan [NAWMP]; USFWS and Canadian Wildlife Service, 1994). Most of these species are hunted throughout their range, subject to damage from oil spills, and prone to ingesting lead and plastics, while water pollution in wintering areas renders these birds vulnerable through their consumption of filter-feeding organisms (Peterson & Ellarson, 1978; Mudge & Allen, 1980; Brown & Brown, 1981; Perry et al., 1984; Piatt et al., 1990; Savard, 1990; Bartonek, 1994; Henny et al., 1995; Flint et al., 1997). By-catch in fishing nets is a large source of mortality for most of these species, although this source of mortality has not been reliably quantified (Scott, 1938; Ellarson 1956; Turnbull et al., 1986). Zydelis et al. (2013) estimated that at least 400,000 birds may be killed in gillnets each year, and that pursuit-diving species, such as loons and seaducks, may be the most vulnerable to entanglement (Piatt & Nettleship, 1987; Zydelis et al., 2009). All of these threats emphasize the importance of understanding the biology and behavior of species in order to create effective management plans to address the threats. Examination of the hearing abilities of these poorly-studied species could assist in mediating all types of threats in several ways. Hearing may play a role in how a bird selects foraging or breeding habitat, pursues prey, finds mates and offspring, or navigates during migration. A deeper understanding of these behaviors will aid in creating management plans to address a suite of potential threats to declining species.

In particular, knowledge of hearing abilities can be used to inform potential impacts of man-made noise sources on aquatic birds. Most current mandates addressing climate change include the construction and expansion of alternative energy sources, such as offshore wind farms. European studies of impacts of wind farms located several kilometers from the coast revealed evidence of avoidance of wind farm areas by loons, gannets, ducks and geese (Petersen et al., 2004; Desholm &

Kahlert, 2005). Avoidance of the area could be due to the visual presence of the turbines or the noise (both in-air and underwater) produced by the wind farm. Not only does the construction of wind turbines increase local underwater noise levels through activities such as pile driving and shipping of materials, but operational noise, while at a much lower level, can affect the surrounding area for the life of the facility (Maxon, 2000; Henriksen, 2001). Noises such as those produced from wind farms (or other sources in the air or underwater) can effect aquatic animals by masking communication or soundscape cues, causing displacement from foraging or breeding areas, or causing physiological damage to auditory and non-auditory tissues ((McCauley et al., 2003; Smith et al., 2004; Wysocki et al., 2006, 2007; Parks et al., 2012; Noren et al., 2012; Casper et al., 2013; Tyack & Janik, 2013).

The majority of studies addressing the impact of noise on aquatic life neglect impacts to birds. Most diving bird species may not dive to depths comparable to marine mammals and fish, but they are exposed to man-made noise sources in the air and underwater, and could potentially be affected.

Some aquatic bird species may be especially susceptible to the impacts of in-air man-made noise sources. For example, some aquatic bird species, such as the northern gannet and common eider, are colony nesters, and in consequence may be very susceptible to noise disturbance. In North America, there are only six colonies for the northern gannet, with hundreds of nests at each location (Mowbray, 2002). When a population is concentrated in several small areas (instead of distributed across a wide range), the population is more susceptible to catastrophic damage as a result of human intrusion at these limited locations (Carnet & Sydeman, 1999; Sladen &

Leresche, 1970; Wilson et al., 1991). Human disturbance in the form of aircraft or visitors caused lowered productivity of cormorant colonies (Kury & Gochfield, 1975), a decline in a breeding population of northern gannets in Ontario (Nettleship, 1975), and desertion rates of 20%-30% in an Adelie penguin colony (Sladen & Leresche, 1970). Severe disturbance from noise sources could potentially cause abandonment of an entire colony.

Other species in this study that may be particularly susceptible to man-made noise disturbance are the two species with the most sensitive hearing (the ruddy duck and the lesser scaup). These two species of diving duck spend the most time in populated areas because of their abundance in coastal waters and inland freshwater bodies (Austin et al., 1998; Brua, 2002). Particular attention should be paid to effects of noise on these species because of their sensitive hearing and frequent exposure to man-made noises such as farming noise, traffic noise, boat noise, and construction.

All bird species studied spend the majority of their time in coastal areas (with the exception of some northern gannets and red-throated loons), within a few kilometers of the coastline (Bureau of Ocean Energy Management, 2013). Man-made noise sources (both in-air and underwater) are often concentrated along the coastline, such as those associated with coastal development and construction, shipping, and recreational boating. In addition, the long-distance migratory behavior of many of these species renders their populations especially susceptible to any stressor that may impede their migratory progress. All of the species researched migrate from breeding areas in Canada and Alaska, as far north as the Arctic Circle, to wintering grounds as far south as Florida (Daniel & Savard, 2011). A noise source (or any human disturbance) that causes a bird to alter its migratory route out of avoidance could cause increased energy expenditure if birds have to fly further than normal (Drewitt & Langston, 2006). To make such a taxing migration, periodic rests at stop-over points along the route are critical for survival. Any disruption or displacement by noise (in-air or underwater) at these stop-over points can cause already weakened birds to endure extra stress and losses in refueling that can affect their chances of reaching their wintering grounds (Drewitt & Langston, 2006). While any one of these impacts (avoidance of a single noise source, displacement from one foraging area, etc.) may not have a population-level effect, the cumulative impacts of aquatic noise, along with other human actions, should be considered when assessing migratory bird fitness and correlations with population effects (Belisle & St. Clair, 2001; Drewitt & Langston, 2006; Masden et al., 2009).

These results provide the first measurements of underwater hearing in any bird. The wide range in diving behavior and physiology across all diving bird species suggest the importance of replicating studies like this to include other diving birds. Further behavioral, physiological and anatomical investigations should be conducted to elucidate the sensitivity and mechanism of hearing in diving birds. This research supported the use of both methods to obtain hearing sensitivity measurements in diving bird species. Although the ABR did not necessarily provide a reliable measure of absolute auditory sensitivity, it allowed for the measurement of bird species that are not kept in captivity anywhere. The efficiency and transportability of the ABR system make it a very useful tool to take advantage of opportunities in which a wild animal is temporarily captured. However, results from this research also emphasize the importance of using psychoacoustic methods to extend our knowledge of underwater hearing in animals that are vulnerable to human impacts in many regards.

LITERATURE CITED

Afton, A. D., & Ankney, C. D. (1991). Nutrient-reserve dynamics of breeding lesser scaup: a test of competing hypotheses. *Condor*, *93*, 89-97.

Au, W.W.L., & Hastings, M.C. (2008). *Principles of marine bioacoustics*. New York: Springer.

Aubin, T., Jouventin, P., & Hildebrand, C. (2000). Penguins use the two-voice system to recognize each other. *Proceedings of the Royal Society B,* 267, 1081-1087.

Austin, J.E., Custer, C.M., & Afton, A.D. (1998). Lesser scaup (*Aythya affinis*). In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxyum.researchport.umd.edu/bna/species/338 doi:10.2173/bna.338

Barr, J.F., Eberl, C., & Mcintyre, J.W. (2000). Red-throated loon (*Gavia stellata*). In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/513

Bayne, E. M., Habib, L., & Boutin, S. (2008). Impacts of chronic anthropogenic noise from energy‐sector activity on abundance of songbirds in the boreal forest. *Conservation Biology*, *22*(5), 1186-1193.

Bélisle, M., & Cassady-St Clair, C. (2002). Cumulative effects of barriers on the movements of forest birds. *Conserv. Ecol.*, *5*(2), 9.

Blickley, J. L., Blackwood, D. & Patricelli, G. L. (2012). Experimental evidence for the effects of chronic anthropogenic noise on abundance of greater sage-grouse at leks. *Conserv. Biol. 26*, 461-471

Bordage, D. & Savard, J.P.L. (2011). Black scoter (*Melanitta americana*). In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/177 doi:10.2173/bna.177

Borg, E. (1982). Auditory thresholds in rats of different age and strain. A behavioral and electrophysiological study. *Hear. Res., 8*(2), 101-115.

Borg, W., & Engström, B. (1983). Hearing thresholds in the rabbit: A behavioral and electrophysiological study. *Acta Oto-laryngologica, 95*, 19-26.

Bost, C.A., Putz, K., & Lage, J. (1994). Maximum diving depth and diving patterns of the gentoo penguin *Pygoscelis papua* at the Crozet Islands. *Mar. Orn.,* 22, 238-244.

Brady, C., Petrie, S., Schummer, M., Badzinski, S., Belzile, N., & Chen, Y. W. (2013). Effects of dietary selenium on the health and survival of captive wintering lesser scaup. *Envir. Poll.*, *175*, 8-15.

Brandt, J. F., & Hollien, H. (1969). Underwater hearing thresholds in man as a function of water depth. *J. Acoust. Soc. Am.*, *46*(4B), 893-894.

Bregman A.S. (1990). *Auditory scene analysis: the perceptual organization of sound*. Cambridge, MA: MIT Press.

Brittan-Powell, E.F., Dooling, R.J. (2004). Development of auditory sensitivity in budgerigars (*Melopsittacus undulatus*). *J. Acoust. Soc. Am.*, *115*, 3092-3102.

Brittan-Powell, E.F., Lohr, B., Hahn, D.C., & Dooling, R.J. (2005). Auditory brainstem responses in the Eastern Screech Owl: An estimate of auditory thresholds. *J. Acoust. Soc. Am., 188*(1), 314-321.

Brittan-Powell, E.F., Dooling, R.J., Gleich, O. (2002). Auditory brainstem responses in adult budgerigars (*Melopsittacus undulatus*). *J. Acoust. Soc. Am., 112*(3), 999-1008.

Brittan-Powell, E.F., Dooling, R.J., Ryals, B.M., & Gleich, O. (2010). Electrophysiological and morphological development of the inner ear in Belgian waterslager canaries. *J. Acoust. Soc. Am.*, *118*, 314-321.

Brown, A. (1992). Identification pitfalls and assessment problems: 12. surf scoter. *Br. Birds.*, *85*, 437-439.

Brown, P.W. (1987). Influence of an unidentified epornitic on waterfowl nesting at Jessie Lake, Alberta. *Can. Field-Nat., 101*, 454-456.

Brown, P.W., & Fredrickson, L.H. (1997). White-winged Scoter (*Melanitta fusca*), In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/274 doi:10.2173/bna.274

Brown-Borg, H. M., Beck, M. M., & Jones, T. A. (1987). Origins of peripheral and brainstem auditory responses in the white leghorn chick. *Comp. Biochem. Physiol.A: Physiol.*, *88*(3), 391-396.

Brua, R.B. (2002). Ruddy duck (*Oxyura jamaicensis*). In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/696 doi:10.2173/bna.696

Budelmann, B. U. (1992). Hearing in nonarthropod invertebrates. In D. Webster, A.N. Popper, & R.R. Fay (Eds.), *The evolutionary biology of hearing* (pp. 141-155). New York: Springer.

Bullock, T.H., Grinnell, A.D., Ikezono, E., Kameda, K., Katsuki, Y., Nomoto, M., Sato, O., Suga, N., & Yanagisawa, K. (1967). Electrophysiological studies of central auditory mechanisms in cetaceans. *Zeitschrift fur vergleichednde Phys., 59*, 117-316.

Bureau of Ocean Energy and Management (2013). Determining offshore use by diving marine birds using satellite telemetry. *Environmental studies program*: http://www.boem.gov/uploadedFiles/BOEM/Environmental_Stewardship/Environmental_Studies/Renewable_Energy/AT%2012-02%20Diving%20Birds.pdf

Burkard, R. & Voigt, H.F. (1989). Stimulus dependencies of the gerbil brainstem auditory evoked response (BAER): I. Effects of click level, rate and polarity. *J. Acoust. Soc. Am., 85*, 2514-2525.

Campo, J. L., Gil, M. G. & Davila, S. G. (2005). Effects of specific noise and music stimuli on stress and fear levels of laying hens of several breeds. *Appl. Anim. Behav. Sci., 91*, 75-84.

Carlson, T.J. (2012). Barotrauma in fish and barotrauma metrics. In A. Popper & A. Hawkins (Eds.), *The Effects of Noise on Aquatic Life* (pp. 229-234). Springer New York.

Carney, K.M., & Sydeman, W.J. (1999). A review of human disturbance effects on nesting colonial waterbirds. *Waterbirds: The Int. J. Waterbird Biol., 22*(1), 68-79.

Carr, C.E., Soares, D., Smolders, J., & Simon, J.Z. (2009). Detection of interaural time differences in the alligator. *J. Neurosci., 29*(25), 7978-7990.

Caras, M.L., Brenowitz, E., & Rubel, E.W. (2010). Peripheral auditory processing changes seasonally in Gambel's white-crowned sparrow. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol., 196*(8), 581-99.

Carpenter, J.W. (2013). *Exotic animal formulary*. St. Louis, MO: Elsevier.

Casper, B. M., Halvorsen, M. B., Carlson, T. J., & Popper, A. N. (2012). Barotrauma effects on fishes in response to impulsive pile driving stimuli. *J. Acoust. Soc. Am.*, *132*(3), 2034-2034.

Casper, B. M., Smith, M. E., Halvorsen, M. B., Sun, H., Carlson, T. J., & Popper, A. N. (2013). Effects of exposure to pile driving sounds on fish inner ear tissues. *Comp. Biochem.Physiol. A: Mol.Integ. Physiol.*, *166*(2), 352-360.

Castellote, M., Clark, C.W., & Lammers, M.O. (2012). Acoustic and behavioural changes by fin whales (*Balaenoptera physalus*) in response to shipping and airgun noise. *Biol. Conserv.*, *147*(1), 115-122.

Cederholm, J.M., Froud, K.E., Wong, A.C., Ko, M., Ryan, A.F., & Housely, G.D. (2012). Differential actions of isoflurane and ketamine-based anaesthetics on cochlear function in the mouse. *Hear. Res., 292*(1-2), 71-9.

Chapman, C.J., & Hawkins, A.D. (1973). A field study of hearing in the cod, *Gadus morhua L. J. Comp. Physiol., 85*, 147-167.

Chappell, M.A., Shoemaker, V.H., Janes, D.N., & Bucher, T.L. (1993). Diving behavior during foraging in breeding Adelie penguins. *Ecol.*, *74*(4), 1204-1215.

Christensen-Dalsgaard, J., Brandt, C., Willis, K. L., Christensen, C. B., Ketten, D., Edds-Walton, P., Fay, R.R., Madsen, P.T., & Carr, C. E. (2012). Specialization for underwater hearing by the tympanic middle ear of the turtle, *Trachemys scripta elegans*. *Proc.Roy. Soc. B: Biol. Sci.*, *279*(1739), 2816-2824.

Christensen-Dalsgaard, J., & Elepfant, A. (1995). Biophysics of underwater hearing in the clawed frog, *Xenopus laevis*. *J. Comp. Physiol. A*, 176, 317-324.

Christensen-Dalsgaard, J., Breithaupt, T., & Elepfant, A. (1990). Underwater hearing in the clawed frog, *Xenopus laevis*. *Naturwissenschaften*, *77*, 135-137.

Corfield, J. R., Krilow, J. M., Ligt, M. N. V., & Iwaniuk, A. N. (2013). A quantitative morphological analysis of the inner ear of galliform birds. *Hear. Res.*, *304*(C), 111–127.

Cornsweet, T. N. (1962). The staircase-method in psychophysics. *Am. J. Psych.,* 485-491.

Cranford, T.W., McKenna, M.F., Soldevilla, M.S., Wiggins, S.M., & Shadwick, R.E., (2008). Anatomic geometry of sound transmission and reception in Cuvier's beaked whale (*Ziphius cavirostris*). *Anat. Rec.*, *291*, 353–378.

Cranford, T. W., Krysl, P., & Amundin, M. (2010). A new acoustic portal into the odontocete ear and vibrational analysis of the tympanoperiotic complex. *PloS one*, *5*(8), e11927.

Croxall, J.P., Davis, R.W., & O'Connell, M.J. (1988). Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. *The Condor, 90*, 157-167.

Cutting, K. A., Hobson, K. A., Rotella, J. J., Warren, J. M., Wainwright-de la Cruz, S. E., & Takekawa, J. Y. (2011). Endogenous contributions to egg protein formation in lesser scaup Aythya affinis. *J. Av. Biol.*, *42*(6), 505-513.

Davis, R. I., Qiu, W., & Hamernik, R. P. (2009). Role of the kurtosis statistic in evaluating complex noise exposures for the protection of hearing. *Ear. Hearing.*, *30*(5), 628-634.

DeRuiter, S.L., & Doukara, K.L. (2012). Loggerhead turtles dive in response to airgun sound exposure. *Endang. Spec.. Res.*, *16*(1), 55-63.

Desholm, M. & Kahlert, J. (2005). Avian collision risk at and offshore wind farm. *Biol. Lett., 1*, 296-298.

Dmitrieva, L.P., & Gottlieb, G. (1992). Development of brainstem auditory pathway in mallard duck embryos and hatchlings. *J. Comp. Physiol. A, 171*, 665-671.

Dooling, R.J. (1980). Behavior and psychophysics of hearing in birds. In A.N. Popper, & R.R. Fay (Eds.), *Comparative studies of hearing in vertebrates* (261-288). New York: Spring-Verlag.

Dooling, R.J. (1982). Auditory perception in birds. In D.E. Kroodsma & E.H. Miller (Eds.), *Communication in birds, Vol 1* (95-130). New York: Academic Press.

Dooling, R.J. (1992). Hearing in birds. In D.B. Webster, R.R. Fay, & A.N. Popper (Eds.), *The evolutionary biology of hearing* (545-559). New York: Springer-Verlag.

Dooling, R. J. (2002). *Avian hearing and the avoidance of wind turbines*. Colorado: National Renewable Energy Laboratory.

Dooling, R.J. & Okanoya, K. (1995). The method of constant stimuli in testing auditory sensitivity in small birds. In G.M. Klump, R.J. Dooling, R.R. Fay, & W.C. Stebbins (Eds.), *Methods in comparative psychoacoustics*. Switzerland: Birkhauser Verlag Basel.

Dooling, R.J. & Saunders, J.C. (1975). Hearing in the parakeet (*Melopsittacus undulatus*): Absolute thresholds, critical ratios, frequency difference limens, and vocalizations. *J. Comp. Physiol. Psych.*, *88*(1), 1-20.

Dooling, R.J., Lohr, B., & Dent, M.L. (2000). Hearing in birds and reptiles. In R.J. Dooling, A.N. Popper, & R.R. Fay (Eds.), *Comparative hearing: Birds and reptiles* pp 308-359. New York, NY: Springer-Verlag.

Drewitt A.L. & Langston, R.H.W. (2006). Assessing the impacts of wind farms on birds. *Ibis*, *148*, 29-42.

Dyson, M.L., Klump, G.M., & Gauger, B. (1998). Absolute hearing thresholds and critical masking ratios in the European barn owl: A comparison with other owls. *J. Comp. Physiol. A., 182*, 695-702.

Early, S. J., Mason, C. R., Zheng, L., Evilsizer, M., Idrobo, F., Harrison, J. M., & Carney, L. H. (2001). Studies of binaural detection in the rabbit (*Oryctolagus cuniculus*) with Pavlovian conditioning. *Behav. Neurosci.*, *115*(3), 650.

Ehrenstein, W. H., & Ehrenstein, A. (1999). Psychophysical methods. In U. Windhorst & H. Johansson (Eds.), *Modern techniques in neuroscience research* (pp. 1211-1241). Springer Berlin Heidelberg.

Ellarson, R. S. (1956). *A study of the old-squaw duck on Lake Michigan*. Ph.D. thesis. Univ. of Wisconsin, Madison.

Fay, R.R. (1988). *Hearing in vertebrates: a psychophysics data book*. Winnetka, IL: Hill-Fay Associates.

Fay, R. R. (1995). Psychoacoustical studies of the sense of hearing in the goldfish using conditioned respiratory suppression. In Klump, G.M. (Ed.), *Methods in comparative psychoacoustics* (pp. 249-262). Birkhäuser Basel.

Fay, R. R., & Popper, A. (2000). Evolution of hearing in vertebrates: the inner ears and processing. *Hear. Res.*, *149*(1-2), 1–10.

Fechner, G. (1966). *Elements of psychophysics.* Vol. I. New York: Holt, Rinehart and Winston.

Finneran, J.J., & Hastings, M.C. (2000). A mathematical analysis of the peripheral auditory system mechanics in the goldfish (*Carassius auratus*). *J. Acoust. Soc. Am., 108*, 3035-3043.

Finneran, J.J. & Hastings, M.C. (2004). A continuous-wave ultrasound system for displacement and amplitude and phase measurement. *J. Acoust. Soc. Am., 115*, 3202-3209.

Finneran, J. J., & Schlundt, C. E. (2004). *Effects of intense pure tones on the behavior of trained odontocetes* (No. TR-1913). SPACE AND NAVAL WARFARE SYSTEMS CENTER SAN DIEGO CA.

Fleischer, G. (1973). On structure and function of the middle ear in the bottlenosed dolphin (*Tursiops truncatus*). *Ninth Annual Conference on Biological Sonar and Diving Mammals*. Menlo Park, CA: Stanford Research Institute Press. pp. 137–179.

Fletcher, H. (1940). Auditory patterns. *Rev. Mod. Phys., 12*, 47-56.

Flint, P. L., Petersen, M.R., & Grand, J.B. (1997). Exposure of spectacled eiders and other diving ducks to lead in western Alaska. *Can. J. Zool*., *75*, 439-443.

Francis, C. D., Ortega, C. P., & Cruz, A. (2009). Noise pollution changes avian communities and species interactions. *Curr. Biol.*, *19*(16), 1415-1419.

Gall, M.D., Brierley, L.E., & Lucas, J.R. (2011). Species and sex effects on auditory processing in brown-headed cowbirds and red-winged blackbirds. *Anim. Behav., 81*(5), 973-982.

Gaston, A.J. (2004). *Seabirds: A natural history*. New Haven, CT: Yale University Press.

Gerstein, E. R., Gerstein, L., Forsythe, S. E., & Blue, J. E. (1999). The underwater audiogram of the West Indian manatee (*Trichechus manatus*). *J. Acoust. Soc. Am.*, *105*(6), 3575-3583.

Gescheider, G. A. (2013). *Psychophysics: the fundamentals*. London: Psychology Press.

Gleich, O., Dooling, R.J., & Manley, G.A. (2005). Audiogram, body mass, and basilar papilla length: Correlations in birds and predictions for extinct archosaurs. *Naturwissenschaften*.

Goold, J. C. (1996). Acoustic assessment of populations of common dolphin *Delphinus delphis* in conjunction with seismic surveying. *J. Mar Biol. Ass. U,K.*, *76*(03), 811-820.

Gorga, M.P., Kaminski, J.R., Beauchaine, K.A., & Jesteadt, W. (1988). Auditory brainstem responses to tone bursts in normally hearing subjects. *J. Speech. Lang. Hear. R.*, *31*, 87-97.

Goudie, R., Gregory, I., Robertson, J., & Reed, A. (2000). Common eider (*Somateria mollissima*), In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/546 doi:10.2173/bna.546

Green, D.M., & Swets, J.A. (1966). *Signal detection theory and psychophysics*. New York: Wiley.

Habib, L., Bayne, E. M., & Boutin, S. (2007). Chronic industrial noise affects pairing success and age structure of ovenbirds Seiurus aurocapilla. *J. App. Ecol.*, *44*(1), 176-184.

Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C., Braun, E. L., Braun, M. J., ... & Yuri, T. (2008). A phylogenomic study of birds reveals their evolutionary history. *Science*, *320*(5884), 1763-1768.

Hall, J.D. & Johnson, C.S. (1971). Auditory thresholds of a killer whale. *J. Acoust. Soc. Am., 51*, 515-517.

Hall, J. W. (1992). *Handbook of auditory evoked responses*. Needham Heights, MA: Allyn & Bacon.

Hamernik, R. P., Ahroon, W. A., Patterson Jr, J. H., & Qiu, W. (2002). Relations among early postexposure noise-induced threshold shifts and permanent threshold shifts in the chinchilla. *J. Acoust. Soc. Am.*, *111*(1), 320-326.

Hawkins, A.D., & Johnstone, A.D.F. (1978). The hearing of the Atlantic salmon, *Salmo salar. J. Fish. Biol., 13*, 665-673.

Heffner, H. E., Koay, G., Hill, E. M., & Heffner, R. S. (2013). Conditioned suppression/avoidance as a procedure for testing hearing in birds: The domestic pigeon (*Columba livia*). *Behav. Res. Methods*, *45*(2), 383-392.

Hemilä, S., Nummela, S., & Reuter, T. (1999). A model of the odontocete middle ear. *Hear. Res.*, *133*, 82–97.

Hemilä, S., Nummela, S., & Reuter, T. (2001). Modeling whale audiograms: effects of bone mass on high-frequency. *Hear. Res.*, *151*, 221–226.

Hemilä, S., Nummela, S., Berta, A., & Reuter, T. (2006). High-frequency hearing in phocid and otariid pinnipeds: An interpretation based on inertial and cochlear constraints. *J. Acoust. Soc. Am.*, *120*(6), 3463-3466.

Henny, C.J., Rudis, D.D., Roffe, T.J., & Robinson-Wilson, E. (1995). Contaminants and sea ducks in Alaska and the circumpolar region. *Environ. Health Perspect.*, *130*, 41-49.

Henriksen, O. (2001). Noise from offshore wind turbines — effects on porpoises and seals. M*Sc thesis, Syddansk Uni- versitet, Odense.*

Henry, K.S., & Lucas, J.R. (2008). Coevolution of auditory sensitivity and temporal resolution with acoustic signal space in three songbirds. *Anim. Behav.*, *76*, 1659-1671.

Henry, K.S., & Lucas, J.R. (2010). Auditory sensitivity and the frequency selectivity of auditory filters in the Carolina chickadee, *Poecile carolinensis. Anim. Behav.*, *80*(3), 497-507.

Higgs, D.M., Brittan-Powell, E.F., Soares, D., Souza, M.J., Carr, C.E., Dooling, R.J., & Popper, A.N. (2002). Amphibious auditory responses of the American alligator. *J. Comp. Physiol. A*, *188*, 217-223.

Higgs, D. M., Lu, Z. & Mann, D. A. (2006). Hearing and mechanoreception. In D.H. Evans & J.B. Claiborne (Eds.), The physiology of fishes. Boca Raton, FL: Taylor & Francis Group.

Hienz, R. D., Sinnott, J. M., & Sachs, M. B. (1977). Auditory sensitivity of the Redwing Blackbird and the Brown-headed Cowbird. *J. Comp. Physiol. Psych.,* *91*, 1365-1376.

Hollien, H., & Brandt, J. F. (1969). Effect of air bubbles in the external auditory meatus on underwater hearing thresholds. *J. Acoust. Soc. Am.*, *46*(2B), 384-387.

Houser, D.S. (2006). A comparison of underwater hearing sensitivity in bottlenose dolphins (*Tursiops truncatus*) determined by eletrophysiological and behavioral methods. *J. Acoust. Soc. Am*, *120*(3), 1713-1722.

Houser, D.S. & Finneran, J.J. (2006). A comparison of underwater hearing sensitivity in bottlenose dolphins (*Tursiops truncatus*) determined by eletrophysiological and behavioral methods. *J. Acoust. Soc. Am.*, *120*(3), 1713-1722.

Hunt, G.L., Harrison, N.M., & Piatt, J.F. (1993). Foraging ecology as related to the distribution of planktivorous auklets in the Bering Sea. In K. Vermeer, K.T. Briggs, K.H. Morgan, & D. Siegel-Causey (Eds.), *The status, ecology, and conservation of marine birds of the North Pacific* (pp. 18-26). Ottawa: Can. Wildl. Ser. Spec. Publ.

Jacobs, D.W. & Tavolga, W.N. (1967). Acoustic intensity limens in the goldfish. *Anim. Behav., 15*, 324-335.

Jacobs, D.W., & Hall, J.D. (1972). Auditory thresholds of a fresh water dolphin (*Inia geoffrensis*) Blainville. *J. Acoust. Soc. Am., 51*, 530-533.

Javel, E., Walsh, E. J., & McGee, J. D. (1986). Development of auditory evoked potentials. *Advances in neural and behavioral development*, *2*, 119-154.

Jensen, K.K. & Klokker, S. (2006). Hearing sensitivity and critical ratios of hooded crows (*Corvus corone cornix*). *J. Acoust. Soc. Am., 119*(2), 1269-1276.

Jewett, D. (1970). Volume-conducted potentials in response to auditory stimuli

as detected by averaging in the cat. *Electroencephalography and Clinical Neurology, 28*, 609-618.

Jewett, D. L. & Williston, J. S. (1971). Auditory-evoked far fields averaged from the scalp of humans. *Brain, 94*, 681-696.

Johnsgard, P.A. (1965). *Handbook of waterfowl behavior*. Ithaca: Comstock Publishing Associates (Cornell University Press).

Johnson, S.C. (1967). Sound detection threshold in marine mammals. In W. Tavolga, (Ed.), *Marine bioacoustics* (247-260). New York: Pergamon Press.

Jouventin, P. (1982). Visual and vocal signals in penguins, their evolution and adaptive characters. *Adv. Ethol., 24*, 1-149.

Jouventin, P., & Aubin, T. (2002). Acoustic systems are adapted to breeding ecologies: individual recognition in nesting penguins. *Anim. Behav.*, *64,* 747-757.

Kalloniatis, M., & Luu, C. (2005). Principles of vision. In H. Kolb, E. Fernandez, & R. Nelson (Eds.),. *Webvision: The rrganization of the retina and visual system* [Internet]. Salt Lake City (UT): University of Utah Health Sciences Center; 1995-. Available from: http://www.ncbi.nlm.nih.gov/books/NBK11513/

Kastak, D.A. (1996). *Comparative aspects of hearing in pinnipeds*. U.C. Santa Cruz: PhD dissertation.

Kastak, D., & Schusterman, R.J. (1999). In-air and underwater hearing sensitivity of a northern elephant seal (*Mirounga angurostris*). *Can. J. Zool.*, *77*, 1751-1758.

Kastak, D.A. & Schusterman, R.J. (2002). Changes in auditory sensitivity with depth in a free-diving California sea lion (*Zalophus californianus). J. Acoust. Soc. Am., 112,* 329-333.

Kastelein, R.A., Dubbeldam, J.L., de Bakker, M.A.G., & Gerrits, N.M. (1996). The anatomy of the walrus head (*Odobenus rosmarus*). Part 4: The ears and their function in aerial and underwater hearing. *Aquatic Mammals*, *22*.2, 95-125.

Kastelein, R. A., Bunskoek, P., Hagedoorn, M., Au, W. W., & de Haan, D. (2002). Audiogram of a harbor porpoise (*Phocoena phocoena*) measured with narrow-band frequency-modulated signals. *The Journal of the Acoustical Society of America*, *112*(1), 334-344.

Kastelein, R. A., Hagedoorn, M., Au, W. W., & de Haan, D. (2003). Audiogram of a striped dolphin (*Stenella coeruleoalba*). *J. Acoust. Soc. Am., 113*(2), 1130-1137.

Kastelein, R. A., van Schie, R., Verboom, W. C., & de Haan, D. (2005). Underwater hearing sensitivity of a male and a female Steller sea lion (*Eumetopias jubatus*). *J. Acoust. Soc. Am.*, *118*(3), 1820-1829.

Katayama, A. (1985). Postnatal development of auditory function in the chicken revealed by auditory brain-stem responses (ABRs). *Electroencephalography and*

Clinical Neurophysiology, 62, 388-398.

Keevin, T.M., & Hempen, G.L. (1997). *The environmental effects of underwater explosions with methods to mitigate impacts*. St. Louis, MO: US Army Corps of Engineers..

Ketten, D.R. (1994). Functional analyses of whale ears: Adaptations for underwater hearing. *IEEE Oceans Proceedings.* Brest, France: 264-270.

Ketten, D.R. (1997). Structure and function in whale ears. *Bioacoust.*, *7*, 103-135.

Ketten, D.R. (2000). Cetacean ears. In W.W.L. Au, A.N. Popper, & R.R. Fay (Eds.), *Hearing by whales and dolphins* (pp. 43-108). New York: Springer-Verlag.

Konishi, M. (1973). Development of auditory neuronal responses in avian embryos. *Proc. Natl. Acad. Sci. USA.*, *70*(6), 1795–1798

Konishi, M. (1973). Locatable and nonlocatable acoustic signals for barn owls. *Am. Nat, 107*, 775-785.

Kooyman, G.L., Cherel, Y., Le Maho, Y., Croxall, J.P., Thorson, P.H., Ridoux, V., &

Kooyman, C.A. (1992). Diving behavior and energetics during foraging cycles in king penguins. *Ecol. Mon., 62*(1): 143-163.

Köppl, C., & Gleich, O. (2007). Evoked cochlear potentials in the barn owl. *Journal of Comparative Physiology a, Sensory, Neural, and Behavioral Physiology*, *193*(6), 601–612. doi:10.1007/s00359-007-0215-0

Köppl, C., Wegscheider, A., Gleich, O., & Manley, G. (2000). A qualitative study of cochlear afferent axons in birds. *Hear. Res., 139*, 123-143.

Kury, C.R., & Gochfield, M. (1975). Human interference and gull predation in cormorant colonies. *Biol. Conserv.*, *8*, 23-34.

Ladich, F., & Fay, R. R. (2013). Auditory evoked potential audiometry in fish. *Reviews in Fish Biology and Fisheries*, *23*(3), 317-364.

Langemann, U., Gauger, B., & Klump, G. M. (1998). Auditory sensitivity in the great tit: perception of signals in the presence and absence of noise. *Anim. Beh.*, *56*(3), 763-769.

Lauer, A.M., Dooling, R.J., & Leek, M.R. (2009). Psychophysical evidence of damaged active processing mechanisms in Belgian Waterslager canaries. *J. Comp. Physiol. A, 195*, 193-202.

Le Prell, C. G. (2012). Noise-induced hearing loss: from animal models to human trials. In A.N. Popper & A. Hawkins (Eds.), *The Effects of Noise on Aquatic Life* (pp. 191-196). Springer New York.

Lengagne, T., Aubin, T., Jouventin, P., & Lauga, J. (2000). Perceptual salience of individually distinctive features in the calls of adult king penguins. *J. Acoust. Soc. Am.*, *107*, 508-516.

Levitt, H. (1970). Transformed up-down methods in psychoacoustics. *J. Acoust. Soc. Am., 49*, 467-477.

Lillis, A., Eggleston, D.B., & Bohnenstiehl, D.R. (2013). Oyster larvae settle in response to habitat-associated underwater sounds. *PLOS One, 8*(10), e79337. doi:10.1371/journal.pone.0079337

Lohr, B., Brittan-Powell, E.F., & Dooling, R.J. (2013). Auditory brainstem response and auditory thresholds in woodpeckers. *J. Acoust. Soc. Am., 133*(1), 337-342.

Lombard, R. E., Fay, R. R., & Werner, Y. L. (1981). Underwater hearing in the frog, *Rana catesbeiana*. *J. Exp. Biol.*, *91*(1), 57-71.

Machin, K.L. (2004). Waterfowl anesthesia. *Seminars in Avian and Exotic Pet Medicine, 13*(4), 206-212.

Machin, K.L., & Caulkett, N.A. (1998). Effects of injectable anesthetics in mallard ducks (*Anas platyrhynchos*): A descriptive study. *J. Avian. Med. Surg., 12,* 255-262.

Maiorana, V.A. & Schleidt, W.M. (1972). The auditory sensitivity of the turkey. *J. Audit. Res.,* 12, 203-207.

Manley, G. A. (2010a). The origin and evolution of high-frequency hearing in (most) mammals. *Hear. Res.*, *270*(1-2), 2–3.

Manley, G. A. (2010b). An evolutionary perspective on middle ears. *Hear. Res.*, 263, 3-8.

Mann, D. A., Lu, Z., Hastings, M. C., & Popper, A. N. (1998). Detection of ultrasonic tones and simulated dolphin echolocation clicks by a teleost fish, the American shad (*Alosa sapidissima*). *J. Acoust. Soc. Am.*, *104*(1), 562-568.

Markov, V.I. (1977). Underwater sounds in Macaroni penguins. In *Adaptations of penguins (*111-121). Moscow: Nauka.

Maruska, K. P., Boyle, K. S., Dewan, L. R. & Tricas, T. C. (2007). Sound production and spectral hearing sensitivity in the Hawaiian sergeant damselfish, *Abudefduf abdominalis*. *J. Exp. Biol.* 210, 3990-4004.

Masden, E. A., Haydon, D. T., Fox, A. D., Furness, R. W., Bullman, R., & Desholm, M. (2009). Barriers to movement: impacts of wind farms on migrating birds. *ICES Journal of Marine Science: Journal du Conseil*, *66*(4), 746-753.

Maxon, C. (2000). *Offshore wind-turbine construction — off- shore pile-driving underwater and above-water noise measurements and analysis*. Salzbergen: Report to SEAS Distribution, Haslev and Enron Wind.

McCauley R.D., Fewtrell, J., Popper, A.N. (2003). High-intensity anthropogenic sound damages fish ears. *J. Acoust. Soc. Am., 113*, 638–42.

McClure, C. J., Ware, H. E., Carlisle, J., Kaltenecker, G., & Barber, J. R. (2013). An experimental investigation into the effects of traffic noise on distributions of birds: avoiding the phantom road. *Proc. Roy. Sci. B.*, *280*(1773), 20132290.

McCormick, J.G., Wever, E.G., Palin, J., & Ridgway, S.H. (1970). Sound conduction in the dolphin ear. *J. Acoust. Soc. Am*., *47*, 1417-1427.

Middlebrooks, J. C., & Green, D. M. (1991). Sound localization by human listeners. *Ann. Rev. Psych.*, *42*(1), 135-159.

Miller, J. D. (1964). Auditory sensitivity of the chinchilla in quiet and in noise. *J. Acoust. Soc. Am., 36*(A), 2010 (1964).

Miller, J. D. (1970). Audibility curve of the chinchilla. *J. Acoust. Soc. Am. 48*, 513–523.

Møhl, B., Au, W.W.L., Pawloski, J.L., & Nachtigall, P.E. (1999). Dolphin hearing: Relative sensitivity as a function of point of application of a contact sound source in the jaw and head region. *J. Acoust. Soc. Am., 104*, 3421-3424.

Møhl, B. (1967). Hearing in seals, in *The Behavior and Physiology of Pinnipeds*, R.J. Harrison, R.C. Hubbard, R.S. Peterson, C.E. Rice and R.J. Schusterman, eds. New York: Appleton-Century-Crofts, pp. 172-195.

Møhl, B., and Ronald, K. (1975). The peripheral auditory system of the Harp seal, *Pagophilus groenlandicus*. *Rapp. P.v. Reun. Cons. Int. Explor. Mer.*, 169: 516-523.

Møller, A. R. (1994). Neural generators of auditory evoked potentials. In J. T. Jacobson (Ed.) *Principles and applications in auditory evoked potentials* (pp. 23-46). Boston, MA: Allyn and Bacon.

Montgomery, J. C., Jeffs, A., Simpson, S. D., Meekan, M., & Tindle, C. (2006). Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. *Adv. Mar. Biol.*, *51*, 143-196.

Mowbray, T.B. (2002). Northern gannet (*Morus bassanus*), In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxyum.researchport.umd.edu/bna/species/693 doi:10.2173/bna.693

Mudge, G. P. & Allen, D.S. (1980). Wintering seaducks in the Moray and Dornoch Firths, Scotland. *Wildfowl, 31*,123-130.

Nachtigall, P.E., Au, W.W.L., Pawlowski, J.L., & Moore, P.W.B. (1995). Risso's dolphin (*Grampus griseus*) hearing thresholds in Kaneohe bay, Hawaii. In

R.A. Kastalein, J.A. Thomas, & P.E. Nachtigall (Eds.), *Sensory systems of aquatic mammals* (pp. 49-53). The Netherlands: De-Spiel, Worden.

Nachtigall, P. E., Yuen, M. M., Mooney, T. A., & Taylor, K. A. (2005). Hearing measurements from a stranded infant Risso's dolphin, *Grampus griseus*. *J. Exp. Biol.*, *208*(21), 4181-4188.

Naguib, M., van Oers, K., Braakhuis, A., Griffioen, M., de Goede, P., & Waas, J. R. (2013). Noise annoys: effects of noise on breeding great tits depend on personality but not on noise characteristics. *An. Beh.*, *85*(5), 949-956.

Nakagawa, S., Waas, J.R., & Miyazaki, M. (2001). Heart rate changes reveal that little blue penguin chicks (*Eudytpula minor*) can use vocal signatures to discriminate familiar from unfamiliar chicks. *Behav. Ecol. Sociobiol., 50*, 180-188.

Nettleship, D.N. (1975). A recent decline of gannets, *Morus bassanus*, on Bonaventure Island, Quebec. *Canadian Field-Naturalist*, *89*, 125-133.

Noirot, I. C., Brittan-Powell, E. F., & Dooling, R. J. (2011). Masked auditory thresholds in three species of birds, as measured by the auditory brainstem response. . *J. Acoust. Soc. Am.*, *129*(6), 3445.

Noren, D. P., Dunkin, R. C., Williams, T. M., & Holt, M. M. (2012). Energetic cost of behaviors performed in response to vessel disturbance: one link in the population consequences of acoustic disturbance model. In A. Popper & A.

Hawkins (Eds.), *The Effects of Noise on Aquatic Life* (pp. 427-430). New York: Springer.

Norris, K.S. (1974). *The porpoise watcher*. New York: W. W. Norton. 250 p.

Nowacek, D. P., Johnson, M. P., & Tyack, P. L. (2004). North Atlantic right whales (*Eubalaena glacialis*) ignore ships but respond to alerting stimuli. *Proc. Roy. Soc. London B*., *271*(1536), 227-231.

Nummela, S., Wägar, T., Hemilä, S., & Reuter, T. (1999). Scaling of the cetacean middle ear. *Hear. Res., 133*, 71–81.

Parks, S. E., Johnson, M., Nowacek, D., & Tyack, P. L. (2011). Individual right whales call louder in increased environmental noise. *Biol. Lett.*, *7*(1), 33-35.

Perry, M. C., Ferrigno, F. & Settle, F.H. (1978). Rehabilitation of birds oiled on two mid-Atlantic estuaries. *Proc. Ann. Conf. S.E. Assoc. Fish Wildl. Agenc., 32,* 318-325.

Perry, M. C, Wells-Berlin, A.M., Kidwell, D.M., & Osenton, P.C. (2007). Temporal changes of populations and trophic relationships of wintering diving ducks in Chesapeake Bay. *Waterbirds 30 (Special Publication* 1), 4-16.

Peterson, S. R. & Ellarson, P.R. (1978). p, p'-DDE, polychlorinated biphenyls, and endrin in oldsquaws in North America, 1969-73. *Pestic. Monit. J., 11*, 170-181.

Petersen, I.K., Clausager, I. & Christensen, T.J. (2004). *Bird numbers and distribution on the Horns Rev. offshore wind farm area. Annual status report*

2003. Rønde, Denmark: National Environmental. Research Institute: Report commissioned by Elsam Engineering A/S 2003.

Piatt, J.F., & Nettleship, D.N. (1985). Diving depths of four alcids. *The Auk, 102*, 239-297.

Piatt, J. F. & Nettleship, D.N. (1987) Incidental catch of marine birds and mammals in fishing nets off Newfoundland, Canada. *Mar. Pollut. Bull., 18*, 344–349.

Piatt, J. F., Lensink, C.J., Butler, W., Kendziorek, M., & Nysewander, D.R. (1990). Immediate impact of the Exxon Valdez oil spill on marine birds. *Auk*, *107*, 387-397.

Pijanowski, B. C., Villanueva-Rivera, L. J., Dumyahn, S. L., Farina, A., Krause, B. L., Napoletano, B. M., & Pieretti, N. (2011). Soundscape ecology: the science of sound in the landscape. *BioScience*, *61*(3), 203-216.

Popper, A. N. (1972). Pure‐tone auditory thresholds for the carp, *Cyprinis carpio*. *J. Acoust. Soc. Am.*, *52*(6B), 1714-1717.

Popper, A.N. (1974). The response of the swim bladder of the goldfish (*Carassius auratus*) to acoustic stimuli. *J. Exp. Biol., 60*, 295-304.

Popper, A. N., & Fay, R. R. (2011). Rethinking sound detection by fishes. *Hear. Res.*, *273*(1-2), 25–36.

Ramprashad, F., Money, K.E., & Ronald, K. (1972). The harp seal*, Pagophilus groenlandicus* (Erxlebel, 1777). XXI. The structure of the vestibular apparatus. *Can. J. Zool*., *50*, 1357-1361.

Ramprashad, F. (1975). Aquatic adaptations in the ear of the harp seal *Pagophilus groenlandicus* (Erxleben, 1777). *Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 169*, 102–111.

Reidenberg, J.S. (2007). Anatomical adaptations of aquatic mammals. *Anat Rec 290*: 507–513.

Reppening, C.A. (1972). Underwater hearing in seals: functional morphology. In R.J. Harrison (Ed.), *Functional anatomy of marine mammals* (pp. 307-331). London: Academic Press.

Reijnen, R., & Foppen, R. U. U. D. (2006). Impact of road traffic on breeding bird populations. In *The ecology of transportation: managing mobility for the environment* (pp. 255-274). Netherlands: Springer Netherlands.

Reubhausen, M.R., Brozoski, T.J., & Bauer, C.A. (2012). A comparison of the effects of isofluorane and ketamine anesthesia on auditory brainstem response (ABR) thresholds in rats. *Hear. Res., 287*(1-2), 25-9.

Rheindt, F. E. (2003). The impact of roads on birds: does song frequency play a role in determining susceptibility to noise pollution?. *Journal für Ornithologie*, *144*(3), 295-306.

Richardson, W. J., Greene Jr, C. R., Malme, C. I., & Thomson, D. H. (1995). *Marine mammals and noise*. New York: Academic.

Richardson, W.J., McDonald, T.L., Greene, C.R., Blackwell, S.B., & Streever, B. (2012). Distribution of bowhead whale calls near an oil production island with fluctuating underwater sound. In A.N. Popper & A. Hawkins (Eds.), *The Effects of Noise on Aquatic Life* (pp. 277-280). Springer New York.

Ridgway, S.H. & Carder, D.A. (1997). Hearing deficits measured in *Tursiops truncatus* and discovery of a deaf/mute dolphin. *J. Acoust. Soc. Am.,* 101, 590-593.

Ridgway, S.H., Carder, D.A., Kamolnick, T., Smith, R.R., Schlundt, C.E., & Elsberry, W.R. (2001). Hearing and whistling in the deep sea: Depth influences whistle spectra but does not attenuate hearing by white whales (*Delphinapterus leucas*), (Odontoceti, Cetacea). *J. Exp. Biol.*, *204*(22), 3829-3841.

Rijke, A. M. (1970). Wettability and phylogenetic development of feather structure in water birds. *J. Exp. Biol.*, *52*(2), 469-479.

Robertson, G. J. & Goudie, R.I. (1999). Harlequin duck (*Histrionicus histrionicus*), In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/466

doi:10.2173/bna.466

Robertson, G.J., & Savard, J.P.L. (2002). Long-tailed duck (*Clangula hyemalis*), In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca.* Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/651 doi:10.2173/bna.651

Robinson, D. E., & Watkins, C. S. (1973). Psychophysical methods in modern psychoacoustics. In J.V. Tobias (Ed.), Foundations of modern auditory theory (pp. 99-131). New York: Academic.

Rogers, P. H., & Cox, M. (1988). Underwater sound as a biological stimulus. In J. Atema, R.R. Fay, & A.N. Popper (Eds.), *Sensory biology of aquatic animals* (pp. 131-149). Springer New York.

Römer, H. & Tautz, J. (1991). Invertebrate auditory receptors. In Ito, F. (Ed), *Comparative aspects of mechano-receptor systems*. New York: Springer-Verlag.

Sadè, J., Handrich, Y., Bernheim, J., & Cohen, D. (2008). Pressure equilibration in the penguin middle ear. *Acta Oto-Laryngologica, 128*, 18-21.

Sauerland, M., & Denhardt, G. (1998). Underwater audiogram of a tucuxi (*Sotalia fluviatilis guianensis*). *J. Acoust. Soc. Am., 103*, 1199-1204.

Saunders, S. S., & Salvi, R. J. (1993). Psychoacoustics of normal adult chickens: thresholds and temporal integration. *J. Acoust. Soc. Am., 94*(1), 83-90.

Savard, J.P.L., Bordage, D. & Reed, A. (1998). Surf scoter (*Melanitta perspicillata*). In A. Poole (Ed.), *The birds of North America online, Cornell Lab of Ornithology, Ithaca*. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.proxy-um.researchport.umd.edu/bna/species/363

doi:10.2173/bna.363

Savard, J. P. L. (1990). Population de sauvagine hivernant dans l'estuaire du Saint-Laurent: écologie, distribution et abondance. *Tech. Rep. Ser. no. 89. Can. Wildl. Serv. Quebec Region.* Quebec.

Scharf, B. (1970). Critical bands. In J.V. Tobias (Ed.), Foundations in modern auditory theory, volume 1 (pp. 159-202). Academic: New York.

Schlundt, C.E., Dear, R.L., Green, L., Houser, D.S., & Finneran, J.J. (2007). Simultaneously measured behavioral and electrophysiological hearing thresholds in a bottlenose dolphin (*Tursiops truncatus*). *J. Acoust. Soc. Am., 122*(1), 615.

Schusterman, R.J. (1980). Behavioral methodology in echolocation by marine mammals. In R.G. Busnel & J.F. Fish (Eds.), *Animal sonar systems* (pp. 11-41). New York: Plenum.

Schusterman, R.J., & Moore, P.W.B. (1978). Underwater audiogram of the northern fur seal, *Callorhinus ursinus*. *J. Acoust. Soc. Am., 64*(1), 87.

Schusterman, R. J., Balliet, R. F., & Nixon, J. (1972). Underwater audiogram of the california sea lion by the conditioned vocalization technique1. *Journal of the Experimental Analysis of Behavior*, *17*(3), 339-350.

Scott, W. E. (1938). Old-squaws taken in gill-nets. *Auk*, *55*, 668.

Sea Duck Joint Venture (2012). *Atlantic and Great Lakes sea duck migration study*. Progress Report: http://seaduckjv.org/atlantic_migration_study/atlantic_grlakes_sea_duck_rpt_november2012_compressed.pdf

Silverman, Emily D., J. B. Leirness, D. Saafeld, M. Koneff, & K.D. Richkus. (2012). *Atlantic Coast Wintering Sea Duck Survey, 2008-2011. Summary Report October 2012 (draft).* Laurel, MD: USFWS.

Simpson, S.D., Meekan, M.G., Jeffs, A., Montgomery, J.C., & McCauley, R.D. (2008). Settlement-stage coral reef fish prefer the higher-frequency invertebrate-generated audible component of reef noise. *Anim. Behav., 75*(6), 1861-1868.

Sisneros, J.A., Popper, A.N., Hawkins, A.D., & Fay, R.R. (2015). Auditory evoked potential audiograms compared to behavioral audiograms in aquatic animals.

Skinner, B.F. (1961). *Cumulative record*. New York: Appleton-Century-Crofts.

Slabbekoorn, H. & Ripmeester, E.A.P. (2008) Birdsong and anthropogenic noise: implications and applications for conservation. *Mol. Ecol. 17*, 72–83

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., & Popper, A. N. (2010). A noisy spring: the impact of globally rising underwater sound levels on fish. *Trends in Ecology & Evolution*, *25*(7), 419-427.

Sladen, W.L., & Leresche, R.E. (1970). New and developing techniques in Antarctic ornithology. *Antarctic Ecology, 1*, 585-596.

Smith, M.E. Kane, A. S., & Popper, A. N. (2004) Noise-induced stress response and hearing loss in goldfish (*Carassius auratus). J. Exp. Biol., 207*, 427–435.

Speckman, S.G., Piatt, J.F., & Springer, A.M. (2003). Deciphering the social structure of marbled murrelets from behavioral observations at sea. *Waterbirds, 26*(3), 266-274.

Stebbins, W. C. (1970). Principles of animal psychophysics. In W. Stebbins (Ed.), *Animal psychophysics: The design and conduct of sensory experiments* (pp. 1-19). New York: Springer.

Stenfors, L. E., Sadè, J., Hellström, S., Anniko, M., & Folkow, L. (2000). Exostoses and cavernous venous formation in the external auditory canal of the hooded seal as a functional physiological organ. *Acta oto-laryngologica*, *120*(8), 940-943.

Stenfors, Jacob Sadé, Sten Hellström, Matti Anniko, L. E. (2001). How can the hooded seal dive to a depth of 1000 m without rupturing its tympanic membrane? A morphological and functional study. *Acta oto-laryngologica*, *121*(6), 689-695.

Szymanski, M.D., Bain, D.E., Kiehl, K., Pennington, S., Wong, S. & Henry, K.R. (1999). Killer whale (*Orcinus orca*) hearing: Auditory brainstem response and behavioral audiograms. *J. Acoust. Soc. Am.*, *106*(2), 1134-1141.

Terhune, J.M. & Ronald, K. (1972). The harp seal, *Pagophilus groenlandicus* (Erxleben, 1771), II. The underwater audiogram. *Can. J. Zool., 50*, 385-390.

Thomas, J., Chun, N., Au, W., & Pugh, K. (1988). Underwater audiogram of a false killer whale (*Pseudorca crassidens*). *The Journal of the Acoustical Society of America*, *84*(3), 936-940.

Tougaard, J., Kyhn, L. A., Amundin, M., Wennerberg, D., & Bordin, C. (2012). Behavioral reactions of harbor porpoise to pile-driving noise. In A.N. Popper & A. Hawkins (Eds.), *The Effects of Noise on Aquatic Life* (pp. 277-280). Springer New York.

Trainer, J.E. (1946). *The auditory acuity of certain birds.* Doctoral dissertation. Cornell University. In Fay, R.R. (Ed.), (1988). Hearing in vertebrates: A psychological databook. Winnetka, IL: Hill-Fay Assoc.

Turnbull, R. E., Brakhage, D.H. & Johnson, F.A. (1986). Lesser scaup mortality from commercial trotlines on Lake Okeechobee, Florida. *Proc. Annu. Conf. Southeast. Assoc. Fish Wildl. Agenc., 40,* 465-469.

Tyack, P. L., & Janik, V. M. (2013). Effects of noise on acoustic signal production in marine mammals. In H. Brumm (Ed.), *Animal Communication and Noise* (pp. 251-271). New York: Springer.

Urick, R. J. (1996). *Principles of underwater sound, 3rd edition*. Newport Beach, CA: Peninsula Publishers. 444 pp.

U.S. Fish and Wildilfe Service and Canadian Wildlife Service. (1994). *1994 update to the North American Waterfowl Management Plan.* Washington, D.C.: U.S. Fish and Wildl. Serv. and Can. Wildl. Serv.

Van Dijk, T. (1973). A comparative study of hearing in owls of the family Strigidae. *Neth. J. Zool., 23*, 131-167.

Varanasi, U., & Malins, D.C. (1971). Unique lipids of the porpoise (*Tursiops gilli*): differences in triacylglycerols and wax esters of acoustic (mandibular and melon) and blubber tissues. *Biochimica et Biophysica Acta*, 231, 415-417.

Warren, J. M., Cutting, K. A., Takekawa, J. Y., De La Cruz, S. E., Williams, T. D., & Koons, D. N. (2014). Previous success and current body condition determine breeding propensity in Lesser Scaup: evidence for the individual heterogeneity hypothesis. *The Auk*, *131*(3), 287-297.

Watson, C.S. & Gengel, R.W. (1969). Signal duration and signal frequency in relation to auditory sensitivity. *J. Acoust. Soc. Am., 46*, 989-997.

Webb, J. F., Smith, W. L., & Ketten, D. R. (2006). The laterophysic connection and swim bladder of butterflyfishes in the genus Chaetodon (Perciformes: Chaetodontidae). *Journal of Morphology*, *267*(11), 1338–1355. doi:10.1002/jmor.10480

Welsch, U., & Riedelsheimer, B. (1997). Histophysiological observations on the external auditory meatus, middle, and inner ear of the Weddell seal (*Leptonychotes weddelli*). *J. Morphol.*, *234*(1), 25-36.

Wever, E.G., Herman, P.N., Simmons, J.A., & Hertzler, D.R. (1969). Hearing in the blackfooted penguin, *Spheniscus demerus*, as represented by the cochlear potentials. *Proc. Natl. Acad. Sci. USA, 63*, 676-680.

Wever, E. G., & Vernon, J. A. (1956a). Sound transmission in the turtle's ear. *Proc. Natl. Acad. Sci. USA*, *42*(5), 292–299.

White, M.J. Jr., Norris, J., Ljungblad, D., Baron, K., & di Sciara, G. (1978). Auditory thresholds of two beluga whales (*Delphinapterus leucas*). *HSWRI Tech. Rep., No. 78-109*. San Diego, CA: Hubbs Marine Research Institute.

Wilson, R.P., Culik, B., Danfeld, R., & Adelung, D. (1991). People in Antarctica – how much do Adelie penguins, *Pygoscelis adeliae* care? *Polar Biology*, *11*, 363-370.

Woehler, E. J. (2004). Hearing abilities in Antarctic penguins. *Polarforschung*, *72*(2/3), 95-98.

Wolski, L.F., Anderson, R.C., Bowles, A.E., & Yochem, P.K. (2003). Measuring hearing in the harbor seal (*Phoca vitulina*): Comparison of behavioral and auditory brainstem response techniques. *J. Acoust. Soc. Am*., *113*(1), 629-637.

Woolley, S.M.N., & Rubel, E.W. (1999). High-frequency auditory feedback is not required for adult song maintenance in Bengalese finches. *J. Neuro*., *19*(1), 358-371.

Wysocki, L.E., Ladich, F., & Dittami, J. (2006). Noise, stress, and cortisol secretion in teleost fishes. *Biol. Conserv., 128*, 501–8.

Wysocki, L.E.(2007). Effects of aquaculture production noise on hearing, growth and disease resistance of rainbow trout, *Oncorhynchus mykiss. Aquaculture, 272*, 687–697.

Yan, H. Y. (1998). Auditory role of the suprabranchial chamber in gourami fish. *J. Comp. Physiol. A.*, *183*(3), 325-333.

Yuen, M.M., Nachtigall, P.E., Breese, M., & Supin, A.Y. (2005). Behavioral and auditory evoked potential audiograms of a false killer whale (*Pseudorca crassidens*). *J. Acoust. Soc. Am.*, *118*(4), 2688-2695.

Yunker, M. P., Herman, L. M. (1974). Discrimination of auditory temporal differences of the bottlenosed dolphin and by the human. *J. Acoust. Soc. Am. 56*, 1870–1875.

Žydelis, R., Bellebaum, J., Österblom, H., Vetemaa, M., Schirmeister, B., Stipniece, A., Dagys, M., van Eerden, M. & Garthe, S. (2009). Bycatch in gillnet fisheries—an overlooked threat to waterbird populations. *Biol. Conserv.*, *142*, 1269–1281.

Žydelis, R., Small, C. & French, G. (2013) The incidental catch of seabirds in gillnet fisheries: A global review. *Biol. Conserv., 162,* 76–88.