Acoustic Communication and Possible Use of Echolocation by the Hippopotamus

(*Hippopotamus amphibius*)

by

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ABSTRACT

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Recent genetic, molecular, and fossil evidence provide support of a hippo-whale clade and suggest their ancestors may have been semi-aquatic. This evidence raises questions about the evolution and adaptation of the common hippopotamus (*Hippopotamus amphibius)* to an aquatic environment. This study sought to examine the amphibious nature of the hippopotamus through investigations of their adaptations, communication, and sensory systems to determine possible connections with their close aquatic relatives, the cetaceans. Audio and video recordings were collected of male and female hippos at Disney's Animal Kingdom®, where the zoological context offered unique and controlled conditions to conduct observational and experimental studies. The goals of this research were to review the literature on the evolution and adaptations of hippos, which revealed adaptations that may represent shared derived characters from the common ancestor of hippos and cetaceans. Measurements and descriptions obtained from a recent dissection of an adult female hippo indicated that their vocal folds were similar in orientation and shape to baleen whales and the fold on their neck did not contain fatty tissue. Research investigating the acoustic and behavioral repertoires of hippos demonstrated three main categories of acoustic signals (burst of air, tonal, and pulsed) that were similar to those recorded from wild hippos. The acoustic parameters and behavioral contexts were reported and analyzed, demonstrating 11 distinct signal types within the three categories. The occurrence of acoustic and behavioral signals during social interactions suggests they mediate dominance and submissive interactions. Observational and experimental approaches used to investigate the potential use of echo-ranging by hippos provided three lines of evidence that support this hypothesis. First, two male hippos produced click trains in non-social contexts while searching for carrot bundles underwater. Second, click trains were associated with obtaining carrot bundles and most were produced prior to chewing. Third, the frequency parameters of clicks could be used to detect objects the size of the carrot bundles underwater. The click trains of hippos resembled clicks produced by young dolphins and may be used to detect larger objects, conspecifics, or for navigation in the murky waters they inhabit.

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Chapter 1: Introduction

The common hippopotamus (*Hippopotamus amphibius*) is a non-ruminant member of the even-toed ungulate order Artiodactyla. Hippo populations are distributed throughout sub-Saharan Africa, but their numbers are declining due to habitat loss and poaching (Lewison, 2007; Feldhake, 2005; Oliver, 1993; Kanga et al., 2011). They are the second largest terrestrial mammal species with an average height of 1.5 meters and weight of 1000-3000 kg. They have a relatively long life span of approximately 40 years (Eltringham, 1999; Estes, 1991). Both common hippos and pygmy hippos (*Choeropsis liberiensis*) are considered amphibious species, spending time both in and out of water, but pygmy hippos are smaller, slightly more terrestrial, and confined to forest regions (Eltringham, 1999). Although the terms amphibious and semi-aquatic are often used interchangeably, animals referred to as amphibious require aquatic conditions during some part of their life (Balian et al., 2008). As an amphibious species, hippos depend on permanent sources of water and give birth and nurse in shallow water (Eltringham, 1999; Feldhake, 2005), but they are not as specialized for aquatic living. In contrast, marine mammals have evolved radical specializations for life in the water such as streamlined body morphology (e.g., cetaceans and pinnipeds), physiological adaptations for diving (e.g., diving bradycardia) and specialized sensory systems (e.g., biosonar).

Very little is known about the adaptations of hippos to aquatic living, nor is much known about their sensory systems. Researchers have only speculated that hippos have a good sense of smell, hearing, and vision, both at night and during the day (Estes, 1991). However, hippos have been reported to possess a vomeronasal organ (VNO), which, in other mammals, is important for olfactory communication (Estes, 1991). Hippos are often seen licking and sniffing the anal areas of other individuals, as well as inspecting dung heaps and urine testing on land (Eltringham, 1999; Klingel, 1991). These behaviors are believed to be important for assessing the status (Olivier & Laurie, 1974) or reproductive states of females and males on land and in the water (Estes, 1991). Like other ungulates, male hippos display the flehmen grimace while urine testing and inspecting the anogential region of other individuals (Zapico, 1999). Their nostrils are closed by muscular valves that may be similar to the dolphin's blowhole (Barklow, 1994), or they may be closed as a reflex when submerging (Eltringham, 1999). Since hippos' nostrils are closed by valves, the flehmen grimace may not be needed to close the external nares for access to the VNO, but rather may be a remnant of their ungulate ancestry (Zapico, 1999).

Recent genetic, molecular, and fossil evidence provide support for a hippo-whale clade and confirm that hippos are the closest living relatives of cetaceans (dolphins and whales) (e.g., Geisler & Theodor, 2009; Spaulding et al., 2009). Using this evidence, several studies have suggested that the common ancestors of hippos and cetaceans were semi-aquatic species that adapted to aquatic conditions after searching for food in the water. These findings raise questions about the evolution and adaptation of the common hippo to an aquatic environment. Due to the challenges of observing hippos in murky waters and the dangers of studying them in the wild, little is known about their behavior, communication, and sensory systems. More research is needed to elucidate possible convergent adaptations or shared derived characters from the common ancestor of cetaceans and hippos.

Natural History of the Common Hippo

Hippos are gregarious and herbivorous ungulates that live in herds typically ranging from 10 to 15, but can include more than 150 individuals in the dry season. Groups of hippos, referred to as herds, pods or schools, consist mostly of females, their calves, juvenile or subordinate males, and a single dominant male (Eltringham, 1999; Estes, 1991; Feldhake, 2005; Karstad &

Hudson, 1986). The location and overall group size is primarily determined by the proximity of grazing land and amount of space available in the water (Harrison et al., 2007; Karstad & Hudson, 1986; Olivier & Laurie, 1974).

During the day, hippos spend a majority of their time in slow moving, shallow water that is deep enough to submerge (Eltringham, 1999). They inhabit rivers and lakes because they depend on sources of permanent water for thermoregulation and due to the properties of their skin (Eltringham, 1999; Luck & Wright, 1964; Noirard et al., 2008). Hippos have a thick dermis that is covered by a thin epidermis that is susceptible to water loss and facilitates cooling (Luck & Wright, 1964). They do not have sweat glands, so it is necessary for hippos to spend the daytime hours in the water or mud to prevent water loss (Eltringham, 1999; Luck & Wright, 1964). Hippos also demonstrate behavioral thermoregulation according to seasonal changes in water temperature, spending more time in the sun when water temperatures decrease (Noirard et al., 2008). In addition, hippos have specialized mucous glands that secrete a reddish substance that acts as a sunscreen, antiseptic, and cools the skin (Eltringham, 1999; Luck & Wright, 1964; Saikawa et al., 2004).

At night, hippos spend most of their time grazing, but may also rest on land or back in the water. As nocturnal grazers, hippos follow well-worn trails to feed on land for approximately 5 to 6 hours at night, which is generally a solitary activity (except for females and calves) (Eltringham, 1999; Estes, 1991; Walther, 1984). Hippos generally live within a few kilometers of grazing areas, but will travel further in search of food during the dry season (Eltingham, 1999; Harrison et al., 2007; Olivier & Laurie, 1974). As hippos travel farther from permanent water sources, temporary wallows are used for resting and/or cooling during the day (Eltringham, 1999). During the dry season, food availability decreases and the density of hippo groups increases, which may act as limiting factors for hippo population sizes (Harrison et al., 2007; Olivier & Laurie, 1974).

Hippos are foregut digesters and referred to as pseudo-ruminants because they have a four-chambered stomach (Chapman 1881; Crisp, 1867; Eltringham, 1999), which may compensate for eating low quality food (Cerling et al., 2008) and having an extremely low metabolic rate (Schwarm et al., 2006). Although hippos are considered exclusive grazers and obligate herbivores feeding on grasses, their diets may be more variable than previously thought (Boisserie et al., 2005c; Cerling et al., 2008; Eltringham, 1999). For example, there are a few reports of hippos eating meat (Dudley, 1998) and aquatic vegetation (Mugangu & Hunter, 1992), and a few anecdotal reports of cannibalism (Eltringham, 1999). These observations are limited, but suggest that when resources are low, hippos will occasionally eat other food items to supplement their diet (Harrison et al., 2007; Feldhake, 2005; Eltringham, 1999). Together with their evolutionary history, this evidence may suggest that hippos forage more often in the water, but further observations are needed.

Hippos are polygynous, where males maintain exclusive mating rights with females in the group, and mating occurs in the water (Eltringham, 1999). Age of sexual maturity varies in captivity and the wild, but hippos reach puberty at about 9 years (7-15) for females and 7.5 years (6-13) for males (Eltringham, 1999; Laws & Clough, 1965; Sayer & Rakha, 1974). Females are polyoestrous and give birth to a single calf during seasons with high rainfall when more food is available and hippos have closer access to their food source along the banks of rivers (Laws & Clough, 1965). Females leave the group and move to shallow water to give birth and nurse (Eltringham, 1999; Klingel, 1991). Gestation lasts 240 days (8 months), with approximately 12 months of lactation (Laws & Clough, 1965). There is little sexual dimorphism other than the slightly larger body size of males and the larger size of male canines, which grow continuously throughout life and serve an important function in threat displays and fighting (Estes, 1991). Sex can be assessed visually, and age can be distinguished by body size, dentition (mandible growth, growth of incisors and canines and tooth wear), and size of an individual's eye lens (Laws, 1968).

Group size and composition depends primarily on seasonal changes in the amount of space available in the water. As hippos migrate along rivers in search of water of sufficient depth, different groups come in to contact, often resulting in conflicts between adult males (Karstad & Hudson, 1986; Klingel, 1991; Olivier & Laurie, 1974). The overall group structure is not fixed, and dominant males (bulls) may allow other males access if they act submissively and do not attempt to mate (Klingel, 1991). However, if conflicts arise, males that are expelled from the group may remain solitary or form bachelor groups (Karstad & Hudson, 1986; Klingel, 1991). The bond between female hippos (cows) and their calves is believed to be the most stable social unit, especially between mothers and daughters prior to reaching maturity (Eltringham, 1999, Estes, 1991; Karstad & Hudson, 1986; Klingel, 1991).

Behavior

While in the water, hippos are often observed resting in shallow water and rafting or resting with their heads on the shore or other individuals (Blowers et al., 2009; Olivier & Laurie, 1974). However, hippos are also more social and tend to display more behaviors while in the water, especially in the late afternoon and early evening (Eltringham, 1999; Karstad & Hudson, 1986; Klingel, 1991). Each group appears to have a dominance hierarchy, usually controlled by the dominant male; yet dominance relationships shift as herds disperse or come together during the dry season (Karstad & Hudson, 1986; Olivier & Laurie, 1974). As a result, aggression levels increase (Estes, 1991), especially between dominant males and subadult males (Klingel, 1991; Karstad & Hudson, 1986; Olivier & Laurie, 1974). When fighting occurs, two male hippos begin by performing ritualized behaviors including visual threats such as posturing, splashing and dung showering, but will become more violent if one does not submit. The males will then push each other, bite and slash at the other's flanks, and ultimately the fight may escalate to jaw-to-jaw sparring (clashing their tusks) (Eltringham, 1999; Estes, 1991; Klingel, 1991; Karstad & Hudson, 1986).

In the dry seasons when deeper water and space are limited, the dominant males become very territorial and often experience conflicts with people (Kanga et al., 2011; Oliver, 1993). Territoriality was first documented by Karstad and Hudson (1986) and confirmed by Klingel (1991). Mature males (bulls) frequently control territories of variable sizes for several years, which include sections of rivers or lakes, and sometimes parts of the shore, with exclusive mating rights. However, these territories do not include grazing areas, suggesting the territorial behavior of dominant males solely represents a reproductive strategy for access to females in the water. Male hippos in lakes appear to have more stable territories than those in rivers, due to the difference in seasonal changes in rivers. Males can hold territories for upwards of eight years, despite other males constantly challenging them. Males will abandon their territories if they dry up, yet they will attempt to return if conditions improve, which can result in severe competition between adult males for space (Eltringham, 1999; Klingel, 1991; Karstad and Hudson, 1986).

There are a few reports of infanticide by dominant male hippos, which has been observed mainly during the dry season when more conflicts occur between males (Estes, 1991; Lewison, 1998; Walther, 1984). On some occasions, females will attack the male to protect the infant (Estes, 1991; Walther, 1984). Similar to other species, infanticide is likely to be used as a strategy for increasing reproductive success for males taking over new territories (Lewison, 1998).

Little is known about the social interactions of females, except for the bond between mother and calf (Karstad & Hudson, 1986; Klingel, 1991). A recent study investigated the behavior of a group of captive female hippos to document behaviors and interactions within the social group (Blowers et al., 2009). This study demonstrated that social interactions among females were influenced by kinship, familiarity, and dominance, which may also affect the type and frequency of social behaviors towards certain individuals (Blowers et al., 2009). Barklow (1997) reported that females may crèche (or care for other individuals') calves, suggesting more complex relationships among females, but further research is needed to better understand the interactions and roles of female hippos.

Male and female hippos perform a variety of visual displays that have been reported to serve important functions in dominance and submissive contexts. Perhaps the most commonly produced behavior by both sexes is the dung shower, which involves wagging the tail while spraying dung and/or urine while on land or in the water (Barklow, 1994; Eltringham, 1999; Karstad & Hudson, 1986; Klingel, 1991; Olivier & Laurie, 1974). Although some studies have suggested the dung shower may be used by dominant males to mark the boundaries of territories or for initial fighting assessment, others have suggested it may be related to olfactory communication and/or marking of trails (Eltringham, 1999; Estes, 1991; Karstad & Hudson, 1986; Klingel, 1991; Olivier & Laurie, 1974; Walther, 1984). When confronted by a dominant individual, males and females will sometimes slowly wag their tail (tail paddle) with or without the emission of dung, suggesting this behavior may also serve as a submissive display (Eltringham, 1999; Estes, 1991; Karstad & Hudson, 1986; Olivier & Laurie, 1974).

In addition to the dung shower, other visual displays are used for threats such as a variety of postures and gaping, or yawning with the mouth open to an approximate 150-degrees. Dominant individuals can be observed standing upright with their ears forward, with their heads held high or with an arched neck, if in the water (Karstad & Hudson, 1986; Klingel, 1991). Although all individuals gape or yawn, dominant males demonstrate the most frequent and energetic gapes with their head thrown back and almost perpendicular to the back. This display is believed to be important for displaying canines during agonistic interactions (Barklow, 1994). Younger individuals appear to model these displays, perhaps practicing for later encounters (Estes, 1991; Karstad & Hudson, 1986). Adult females have been reported to "lip-wrestle" where they carefully match their lips while gaping and pushing each other, which was observed to precede mating with a male (Karstad & Hudson, 1991). Defense or submissive displays are demonstrated towards dominant or larger individuals and include open mouth displays, ears hanging down, lifting their hind end out of the water while tail paddling, lying prone, crouching, and flight (Eltringham, 1999; Estes, 1991; Karstad & Hudson, 1986; Klingel, 1991; Olivier & Laurie, 1986).

Vocal and acoustic signals may coincide with specific non-vocal behavior in social contexts. For example, dominant individuals produce big splashes by thrusting their heads up and down in the water and will roar and chase away subordinates or other dominant individuals challenging their territory. Hippos also release a burst of air underwater that produces a large fountain-like effect on the surface, which may serve as a threat display (Barklow, 2004; Estes, 1991). The occurrence of simultaneous vocal, acoustic and non-vocal behavior suggests that the hippo, like other species, may use a variety of multimodal signals for communication. For a more in depth description of hippo behavior, see the ethogram (*Appendix one*).

Vocal and acoustic signals

Only a few studies have described hippo acoustic communication in the field (Barklow, 1994, 1997, 2004) and in captivity (Krueger, 2000; Barklow, 2004), but these studies suggest hippos have a complex communication system. Since it is not yet certain how hippos produce sounds, these signals will be referred to as acoustic signals. Previous studies have reported that hippos produce a variety of underwater and airborne acoustic signals, described as tonal or pulsed, that were correlated with social contexts (Barklow, 1994, 1997, 2004). These signals can be produced either separately or simultaneously in-air and underwater (referred to as "simultaneous amphibious sound" or SAS), which suggests hippos have specific adaptations that enable them to communicate through both mediums (Barklow 1994, 1997, 2004). These SAS call types are produced when hippos are in the "amphibious position" with eyes, ears, and nostrils above the surface and mouth, throat and rest of the body submerged, enabling hippos to communicate with animals that are either in water or on land (Barklow, 1994, 1997, 2004; Eltringham, 1999). To ensure that hippos could receive sounds in both media, Barklow (1994, 1997, 2004) played back hippos sounds and determined they could detect these sounds both above and below the surface based on their orientation responses.

Similar to other ungulates, hippos seem to vocalize primarily under higher excitement levels, which is typically in the late afternoon and early evening before hippos move on land to graze (Barklow, 1994, 1997, 2004; Karstad & Hudson, 1986; Walther, 1984). Hippos produce a variety of grunts, bellows, and squeals that are very loud and travel long distances (Eltringham, 1999; Karstad & Hudson, 1986), but none of these are produced only on land (Barklow, 2004). Hippos also produce exhaled bursts when threatened or alarmed (Karstad & Hudson, 1986). One of the most common sounds observed from hippos in Africa is the wheeze honk, which has been described as a bellow followed by a series of grunts or honks (Estes, 1991; Karstad & Hudson, 1986) produced above and below the surface (SAS) (Barklow 1994, 1997, 2004). This sound is considered a contact call and produced by female and male hippos in a variety of contexts. Usually the dominant individual vocalizes first, followed by other individuals rising to the surface to chorus (Barklow, 1994; Estes, 1991; Feldhake, 2005; Walther, 1984). Since dominant males often wheeze honk first, this signal may also be important for communication across territories (Barklow, 1994). To better understand the relevance of these signals, more information is needed about the social contexts in which these acoustic signals are produced.

The acoustic characteristics of the communication signals have only been described in two studies (Barklow, 1997, 2004). Barklow (1997) identified three categories of underwater sounds: tonal whines, pulsed croaks and clicks. Each category was described in terms of the acoustic parameters (e.g., duration, fundamental frequency, frequency of peak amplitude, etc.) from spectrograms and the behavioral contexts, which were only based on a few observations. In a later study, Barklow (2004) described nine general categories of non-discrete or graded sounds that hippos could produce as SAS signals. These signals had mean peak frequencies below 205 Hz for aerial sounds and below 2087 Hz for underwater sounds. Barklow (1994, 2004) also suggested that hippo vocalizations may have infrasonic components (frequencies below 20 Hz), but recording systems with a broader frequency range were needed to confirm this hypothesis.

Since hippos have well-established social groups and spend time in turbid water, these signals could play an important role in maintaining group cohesion and coordination between these social mammals across long distances. For example, SAS signals facilitate communication between individuals above and below the surface. Due to the rapid speed of sound transmission in water, the underwater components precede the aerial components, which may be an important mechanism for alerting submerged individuals. Additional research is needed to further describe and quantify the type and contextual use of acoustic signals emitted by hippos, especially when they are submerged because this seems to be the context in which they communicate most frequently.

Virtually no research has been conducted on the mechanisms of sound production and reception in hippos, but there are some hypotheses about how sounds are produced and received. Researchers have suggested that some of the aerial components of sounds travel through the snout and out of the nostrils since no bubbles are emitted from the mouth (Barklow, 1994, 1997, 2004). Underwater sounds include little to no expiration of air since the nostrils are closed when hippos are submerged (Estes, 1991; Barklow, 1997). Thus, these observations suggest hippos produce sounds from their larynx, which may be propagated through the large fold of blubber around the throat (Barklow, 1994, 2004; Feldhake, 2005).

The principles of human speech production described by Fant (1960) have been applied to sound production in mammals assuming similar mechanisms are involved (e.g., Fitch, 1997; Taylor & Reby, 2010). The primary vocal organs include the lungs, trachea, larynx and/or vocal folds, pharynx, and nasal and oral cavities (Denes & Pinson, 1993). The typical pathway for sound production begins with the passage of air from the lungs through the larynx, which causes the vocal folds to vibrate and generate sound (e.g., Fitch, 1997; Taylor & Reby, 2010). The rate at which the vocal folds vibrate (measured in Hertz (Hz)) determines the fundamental frequency and harmonics of the sound (Denes & Pinson, 1993; Fant, 1960; Taylor & Reby, 2010). From the vocal folds, the sound travels up through the vocal tract to the oral and nasal cavities. The shape and length of the vocal tract can filter sounds by acting as a resonator, emphasizing certain resonant frequencies that have spectral peaks referred to as formants (Denes & Pinson, 1993;

Fant, 1960; Taylor & Reby, 2010). These principles are the basis for source-filter theory of speech and sound production, which describes two stages of sound production (the source and filter) that determine the acoustic properties of sounds (Fant, 1960; Taylor & Reby, 2010). Source-filter theory can be applied to examine the effects of the source and filter on the acoustic parameters of different sounds.

The physical characteristics and structure of the vocal organs are related to body size, weight, age and sex, and can reveal information about the individual producing sounds (Fitch, 1997; Taylor & Reby, 2010). The size of the vocal folds ("source") affects the rate of vibration, which determines the pitch of sound. For example, larger vocal folds vibrate at a slower rate, resulting in a lower pitch or fundamental frequency (F_0). However, the size of the vocal folds is not a reliable indicator of body size because they are made of soft tissue and not restricted by skeletal structures (Fitch, 1997). In addition, source-filter theory can be applied to investigate how the vocal tract "filters" sounds. The vocal tract is represented as a tube that is closed at one end (as the vocal folds open and close) and open at the other (either mouth or nostrils). Using this model, researchers can predict the location of formants or estimate the length of the vocal tract (e.g., Fitch, 1997; Taylor & Reby 2010).

For sound reception, sound waves generally reach the external ear and enter into the auditory canal. At the end of the ear canal, the sound waves reach the tympanic membrane (or eardrum), causing it to vibrate. Movement of the eardrum is transmitted through the middle ear ossicles (malleus, incus, and stapes) to the oval window of the inner ear. Movement of the oval window creates a pressure wave in the fluid filled cochlea that vibrates the basilar membrane. This motion activates the hair cells in the organ of Corti of the basilar membrane, which convert these vibrations to neural signals that are processed in the central nervous system (Denes & Pinson, 1993).

Hippos may receive sounds in-air similar to the pathways described above. However, hippos fold their ears back when they are submerged to prevent water entry. Thus, researchers have suggested that hippos may have evolved bone conduction and "jaw hearing" to receive sounds underwater, similar to cetacean species (Barklow, 1994, 2004). For sound reception underwater, cetaceans have evolved mechanisms such as jaw hearing, bone conduction and a modified ear that lacks an external pinnae and external auditory meatus, which have been functionally replaced by the mandibles and mandibular fat pad (Ketten, 1997; Nummela et al., 2007). Lack of external ears and bone conduction make sound localization more difficult, but adaptations such as the telescoping of their skulls and head scanning movements may make directional hearing possible and may also be important for echolocation (Nummela et al., 2007). Several studies have demonstrated the importance of the dolphin jaw for hearing (e.g., Brill & Harder, 1991; Potter & Taylor, 2001), which has a thin oval area of fat that sits over a thin portion of the posterior end of the lower jawbone. Sounds pass through this area to the mandibular fat body to the middle and inner ear (Norris, 1968; Koopman et al., 2006). Inspections of hippo skulls revealed that there is a thin disk at the back of the jawbone, which may help them receive sounds in water (Barklow, 1994, 2004; Feldhake, 2005). Further research is necessary to examine the functions and mechanisms of hippopotamus communication, which may also reveal more information about the evolutionary connections between hippos and cetaceans.

Notably, researchers have reported that hippos produce clicks underwater that resemble echolocation signals produced by their close aquatic relatives, the cetaceans (Barklow 1994, 1997, 2004; Kreuger, 2000). Echolocation is recognized as a sophisticated biosonar sensory system found mainly in odontocetes (dolphins and toothed whales) and microchiropteran bats in which the animals emit acoustic signals into their surrounding environment. The sound waves reflect off of objects and produce an echo that is received and processed by the animals to provide an acoustic picture of their surroundings when vision is limited. Other species such as shrews, tenrecs, oil birds, and cave swiftlets use forms of echolocation for navigation or orientation, but their systems may not be as specialized as toothed whales and bats (Buchler & Mitz, 1980; Thomas & Jalili, 2004). Though there is some variation in the characteristics of the pulsed signals used for echolocation, they are typically broadband signals, short in duration that are emitted into the surrounding environment and reflect off objects. Animals rely on the delay in time and differences in the intensity of the returning echo as it reaches the animal's ears for ranging, or determining the direction and distance of objects (Jones, 2005) (*See Chapter 5 for more information*).

In the case of cetaceans, echolocation has enabled them to adapt and survive in a marine environment where it may not be possible to rely on the visual sense (Au & Hastings, 2008; Schusterman et al., 2000). These systems involve concurrent adaptations of the physiological mechanisms for both sound production and reception, resulting in a highly specialized sensory system. Numerous experiments have demonstrated the remarkable specificity of the echolocation system and the amount of detail that can be received and processed from the echoes following clicks produced by individual dolphins (Au & Hastings, 2008).

There is some evidence to suggest that dolphins and several other cetaceans use pulsed signals in a variety of contexts including echolocation (Au & Hastings, 2008). For example, pulsed signals may be used for social communication (Au & Hastings 2008), foraging (e.g.

Stimpert et al. 2009), and other conditions such as single clicks associated with fright (Caldwell et al., 1962), as well as click trains for echolocation. For species such as lesser bulldog bats, echolocation clicks may serve a dual function for both echolocation and communication (Voigt-Heucke et al., 2010). Thus, it is possible that pulsed signals may still have some functional significance in other species that remain to be studied.

Barklow (1997) observed the production of click sequences (click trains) by wild and captive hippos underwater. He recorded several click type signals and described them as wide-band clicks, narrow-band clicks, click trains, and pulsed croaks (Barklow, 1994, 1997, 2004). However, he reported that click train occurrence coincided with aggressive social interactions and suggested that they function as social signals (Barklow, 1997). One study experimentally tested whether click trains might function as echolocation signals in hippos (Kreguer, 2000). In this study, captive hippos were tested at night and in cloudy water to determine whether they would navigate around and avoid large plastic barrels in their pools. The results were compelling in that many clicks were recorded, but there was no difference in the number of clicks during the experiment versus control conditions. As a result, the researchers could not conclude that hippo clicks were used as a form of echolocation (Krueger, 2000).

The question as to whether hippos use clicks as a form of echolocation remains unanswered. Hippos are an amphibious species that give birth and nurse in water and spend much of their time interacting when submerged in the water. As a result, hippos may have developed a form of echo-ranging to be able to detect objects, conspecifics and their young, and potential predators in the murky waters they inhabit. Finding evidence of a form of echo-ranging could reveal information about the evolution of hippos' sensory systems and provide insights about whether echolocation may represent convergent evolution or a shared derived character from the common ancestor of hippos and cetaceans.

Research Goals

This study sought to examine the amphibious nature of the hippopotamus through investigations of their communication systems, adaptations, and sensory systems to determine possible connections with their close aquatic relatives, the cetaceans. Audio and video recordings were collected of male and female hippos at Disney's Animal Kingdom®, where the zoological context offered unique and controlled conditions to conduct observational and experimental studies to further investigate the acoustic signals and sensory systems of hippos. The goals of this study were to: 1) investigate the adaptations of hippos and mechanisms of sound production, 2) describe and quantify the acoustic repertoire of a captive group of male and female hippos, 3) describe the contextual use of the acoustic signals, 4) determine possible sex differences in the use and parameters of their acoustic signals, 5) compare the acoustic signals of this group with published data on the signals of other captive and wild hippos, and 6) test the hypothesis that hippos use a form of echo-ranging.

The following chapters represent the different components of this study. In each chapter, I present the relevant background information, methodology, results, discussion and conclusion. Chapter 2 presents a review of research on the evolution of the hippopotamus, including findings on their aquatic adaptations and sensory organs. Chapter 3 includes a review of the vocal anatomy of hippos and new data from a recent dissection with measurements and descriptions of their vocal organs. Chapter 4 focuses on the acoustic repertoire and contextual use of signals by male and female hippos. Chapter 5 focuses on research investigating whether the click trains produced by hippos may be used for echo-ranging. These chapters are followed by the conclusion section, appendix and bibliography.

Chapter 2: Evolution of the hippopotamus

Hippopotamidae first appeared approximately 15 million years ago during the Miocene period in Africa and parts of Asia and have been classified as part of the order Artiodactyla. Hippos are believed to have descended from Antracotheriidae, a semi-aquatic group that branched off from ruminants and other even-toed ungulates that may have entered the water in search of food (Boisserie et al., 2005a; Boisserie, 2005). Early hippos evolved to an amphibious existence, increasing in size as they occupied a new niche in the water. Hippopotamidae is considered to be a paraphyletic group containing three main genera (as of around 8 mya): *Hippopotamus*, *Hexaprotodon* (mainly Asian species), and *Choeropsis* (Boisserie, 2005).

Researchers have tracked the evolution of hippos, primarily relying on craniodental morphology, which supports the three genera. Most current research has focused on the extant species, which likely survived as they found and adapted to permanent sources of water (Boisserie, 2005; Jablonski, 2004). The extant hippos, which include the common hippo (*Hippopotamus amphibius*) and pygmy hippo (*Choeropsis liberiensis*), are amphibious and require permanent sources of water. The pygmy hippo, described as a forest dweller and a more primitive species (Eltringham, 1999), has at different times been classified as a member of the *Hexaprotodon* group (Boisserie, 2005; Jablonski, 2004). Members of the *Hexaprotodon* genus were believed to have gone extinct due to changes in the environment and the increased seasonality of rainfall during the late Neogene period (Jablonski, 2004). Assuming hippos already required permanent sources of water, the increased seasonality in rainfall resulted in a lack of permanent water, causing a die off of this group (Jablonski, 2004).

Initially, hippos were thought to be closely related to pigs and peccaries (Suidae) until Sarich (1993) presented findings using molecular data that hippos were the closest living relatives of cetaceans. Although there is ongoing debate regarding the placement of these species, discussions about the relationship between hippos and cetaceans have continued with many studies using various measures of molecular, genetic and fossil evidence to provide further support of their evolutionary connection (e.g., Boisserie et al., 2005b; Geisler & Uhen, 2003, 2005; Geisler & Theodor, 2009; Gingerich, et al., 2001; Spaulding et al. 2009; Ursing & Arnasson, 1998). Prior to these findings, cetaceans had been placed within artiodactyls, but there is still much debate about where they fit in this group. Current research has suggested that cetaceans evolved within artiodactyls, with pakicetus (the earliest known whale) first appearing during the Eocene period around 54 million years ago (Uhen, 2007). Thus, questions about whether hippos and cetaceans form a sister group are complicated by the gap in evolutionary history based on their first appearances in the fossil record.

Thewissen and colleagues (2007) investigated the relationship between cetaceans and artiodactyls, presenting fossil data that placed hippos with pigs and peccaries and Raoellidae (considered aquatic waders) as the closest relative to cetaceans. A follow-up study by Geisler and Theodor (2009) used a modified version of their data set that included molecular characters to conclude that hippos were the closest extant relatives to cetaceans, while Raoellids were the closest extinct group. A study by Spaulding and colleagues (2009) analyzed a larger data set of molecular and fossil characters from a wider range of taxa and provided further support for a hippo-whale clade. Despite the discrepancies over the placement of hippos, all of these studies suggested that semi-aquatic living pre-dated the origin of cetaceans and hippos and proposed that changes in diet (e.g., abundance of food in the water) led the ancestors of cetaceans and hippos into the water, as indicated by changes in the shape of their molars (e.g., Spaulding et al., 2009). Spaulding and colleagues (2009) also found that extinct and extant members of this group appeared to share the derived ability to hear underwater, even though many of these species (hippos included) lack pachyostosis (or thickening) of the auditory bulla. This finding changed the view that a pachyostotic auditory bulla was necessary for underwater hearing, but further research is needed to determine whether this may be important for directional hearing (Spaulding et al., 2009). These studies suggest the common ancestor of hippos and whales may have evolved some adaptations for aquatic living, but it remains unclear as to what other adaptations may have existed.

Due to their amphibious nature, hippos represent an interesting case study for revealing the evolutionary changes that occurred as their semi-aquatic ancestors adapted to conditions in the water. The evolutionary connection between hippos and cetaceans allows for comparisons of how these species adapted to aquatic living. What kinds of physiological and sensory adaptations did their common ancestor have? Do hippos and cetaceans show similar or different adaptations to amphibious and aquatic life? In an attempt to resolve some of these questions, I will examine the adaptations of hippos in comparison to the fully aquatic cetaceans to better understand their amphibious lifestyle.

Aquatic adaptations

Hippos have several adaptations indicative of amphibious living, which include partially webbed feet, skin that is mostly hairless and glandular, valvular nostrils and ears, and dorsally protruding nostrils and eyes (Coughlin & Fish, 2009; Eltringham, 1999). They have a tube-shaped body that is not as streamlined as marine mammals (Eltringham, 1999), but may facilitate movement in water. The top layer of their skin is thin and susceptible to water loss, but since they do not have sweat glands, this direct loss of water is believed to be important for cooling their body temperature (Luck & Wright, 1964). In addition, hippos have glands that secrete a red, sunscreen-like substance that protects their skin when exposed out of the water (Luck & Wright, 1964; Saikawa et al., 2004). Common and pygmy hippos can close their nostrils and ears by folding them back when submerged to prevent water entry (Pocock, 1923), which suggests there are valves that may be opened or closed by the hippos (Eltringham, 1999). The dorsal positioning of their eyes and ears enables hippos to remain in the "amphibious position" with their eyes, ears, and nostrils above the surface, but mouth and throat below the surface when resting or remaining alert (Barklow, 1994, 1997, 2004). The amphibious position may be an important adaptation for hippos to maintain some awareness of what is going on above and below the surface.

The amphibious hippo has been described as an excellent diver and swimmer, yet they do not actually swim. Hippos are bottom walkers and can be observed gliding through the water as they push off from the bottom to rise to the surface. Research by Fisher et al. (2007) examined the musculature of the forelimbs of the pygmy hippo and noted that the muscles were increased in size and fused, and they have modified pectoral muscles that may counteract the resistance of water for underwater movement. These structures appear to be similar to those found in common hippos (e.g., Macdonald et al., 1985), and resemble other ruminants rather than pigs or peccaries (Suids) (Fisher et al., 2007). Hippos may be unique among artiodactyls in retaining several primitive structures in their forelimbs, providing further support that hippos diverged earlier from this group (Fisher et al., 2007). Similar to other semi-aquatic species and their possible ancestors or relatives the Raoellids, hippos also have denser bones that aid in bottom walking (Coughlin & Fish, 2009; Geisler & Theodor, 2009; Thewissen et al., 2007), suggesting some of these characters may be inherited from their ancestors.

Coughlin and Fish (2009) examined the movement of hippos underwater and on land and noted some differences. Hippos on land walk with three legs on the ground to support their weight, but underwater hippos use an unstable gait, similar to a gallop, with periods of no contact with the bottom. Hippos use mostly their forefeet to push off the bottom, which is possible due to increased buoyancy and relates to the musculature of their forelimbs and weight distribution of their body (Coughlin & Fish, 2009).

While some reports have suggested that hippos can remain submerged for up to 30 minutes, the average time spent underwater is closer to five or six minutes as it is dependent on how long hippos can hold their breath (Eltringham, 1999; Parker, 1935; Wright, 1972). In mammals, respiration rates at rest generally decrease with increasing body weight, although aquatic animals tend to have lower respiration rates than terrestrial species (Mortola & Limoges, 2006). Observations of captive hippos revealed they often breathe in one to two-minute intervals with a range between 5 seconds and 4 minutes and 40 seconds, and they tend to remain submerged longer when disturbed (Parker, 1935). The typical breathing pattern involves hippos holding their breath, emitting a few bubbles before rising to the surface where they open their nostrils and air rushes out (Eltringham, 1999; Mortola & Limoges, 2006). The respiration rate of hippos is relatively low (2 breaths per minute), which is consistent with their large body weight and amphibious lifestyle, and comparable to dolphins (2.3 breaths per minute). Notably, hippos have been reported to decrease their breathing rates under high water conditions as compared to low water conditions (Mortola & Limoges, 2006).

Although the diving times of hippos are relatively short and limited by the amount of time they can hold their breath, they may demonstrate diving bradycardia, which is the slowing of heart rate in diving animals to compensate for asphyxia (Elsner, 1966). A 6-month old hippo was observed while diving in captivity where it demonstrated a decrease in heart rate, suggesting circulation changes similar to harbour seals (Elsner, 1966). Dissection of the vascular system and vena cava of the heart revealed that hippo blood is highly oxygenated and blood is retained in the viscera and lower extremities, which may enable hippos to remain submerged for long periods without breathing (Chapman, 1881). Although oxygen consumption decreases when hippos are in the water, more recent studies suggest that their blood parameters do not support the hypothesis that they demonstrate diving bradycardia (Feldhake, 2005; Wright, 1973).

Sensory organs

Hippos have been reported to have good vision (diurnal and possibly scotopic), hearing, and smell (Estes, 1991), but no tests have been conducted to investigate these sensory systems. Some studies have described the structures of these organs, but most involve comparisons with other species. For example, hippo eyes share similar adaptations to both terrestrial and aquatic mammals, suggesting their eye structures reflect their amphibious environment (Hatfield et al., 2003; Hegner, 1967; Natiello et al., 2005). Hippos' eyes have increased vascularization in the ciliary body of the eye that was similar, but less prominent than aquatic mammals, suggesting that hippos represent an intermediate aquatic state (Natiello et al., 2005). Like cetaceans, hippo eyes have a tapetum lucidum (Luck, 1965) or tapetum-like structures (Hegner, 1967) that may be important for nocturnal vision. However, marine mammals have only L (long) cones, while hippos have both S (short) and L (long) cones in their eyes, meaning they are likely to have dichromatic vision (Peichl et al., 2001). There have been no tests of visual acuity and there are conflicting reports about how far they can see, yet some researchers have speculated hippos can see objects up to 400 yards away based on their reactions to the approach of people (Solomon, personal communication).

Similarly, studies on the hearing capacities or thresholds of hippos are lacking. Hippos have been confirmed to be capable of receiving sounds in-air and underwater through playback studies (Barklow, 1994, 1997, 2004), which suggests they have adaptations to compensate for the physical properties of sound and impedance in the two different mediums. When submerged, hippos fold their ears back to prevent water from entering, but nothing is known about how they receive these sounds or what frequency range they can detect. Hippos have been suggested to use jaw hearing and/or bone conduction for hearing underwater since their jaw has a thin disk at the back that may be useful for receiving sounds underwater (Barklow, 1994, 2004; Feldhake, 2005). In addition, their middle and inner ear bones are partially suspended from the skull by ligaments that could reduce bone conduction (Barklow, 1994, 2004; Feldhake, 2005). The middle ear of hippos has three attachments: one to the outer ear, one to the skull and attached to jaw, and a third to the cochlea, suggesting that hippos may receive sounds in two ways, in-air through the outer ear or underwater through the jaw (Barklow 1994).

In terms of their sense of smell, hippo possess a vomeronasal organ (VNO) that is used for urine testing, inspecting dung heaps, and anogenital sniffing of other individuals both on land and in the water (Estes, 1991). Hippos may use the VNO to assess the age, sex, dominance, or reproductive status of individuals (Estes, 1991; Olivier & Laurie, 1974). Hippos also demonstrate the flehmen response, common to other ungulates, yet this display may be remnant of their ancestry rather than to access the VNO (Zapico, 1999). Although hippos seal their nostrils when submerged (Eltringham, 1999; Mortola & Limoges, 2006), they may be able to taste or smell through their mouth. In general, further research is needed to better understand how hippos use these sensory organs and other possible sensory systems.

Preliminary observations of captive hippos–nostril state

During the course of this study, the state of the nostrils of male and female hippos was observed at Disney's Animal Kingdom®, which appeared to be closed when they were resting. This observation led to the hypothesis that similar to cetaceans, hippos may be voluntary breathers, meaning they have control over opening their nostrils to breathe. Hippos flare their nostrils when alarmed and while producing sounds, but nothing is known about their normal resting state. Similar to aquatic mammals, it would be expected that hippos would close their nostrils when submerged to prevent water entry. However, if their nostrils were closed when they were resting, including on land, it may suggest that they have control over the state of their nostrils and breathing.

Methods

Over two days in January 2010, opportunistic video recordings (approximately one hour in duration) were collected using a Canon HV20 video camera to observe the state of the nostrils of a male hippo while resting in the inside holding area. These recordings were made while the hippo was interacting with a familiar zookeeper who encouraged the hippo to station (standing) and to lie down (which was part of their husbandry training). Once he was in the relaxed position, the state of the nostrils was recorded. However, the hippo was alerted to my presence, so I moved out of sight and asked the zookeeper to describe the nostrils. To examine the state of the nostrils when hippos were in the water, previously recorded videos taken from above the hippos in the exhibit pools (e.g., the roof or cliff overlooking the pool) were reviewed so that the nostrils were in sight. However, this information could not be quantified due to the movement of the hippo, which meant the nostrils were not always in view.

Results

Visual inspection of the hippos while in a relaxed position (standing or lying down) on land revealed that the nostrils appear to be closed in a relaxed state. However, there were variations in the state of the nostrils, which may be fully open, half or partly open, or closed (Figure 2.1). In the water, hippos can be observed submerging (nostrils closed) and then rising to the surface to breathe in exhaled bursts, followed by inhaling and submerging again. Hippos in the amphibious position were observed with their nostrils open and closed (Figure 2.2).

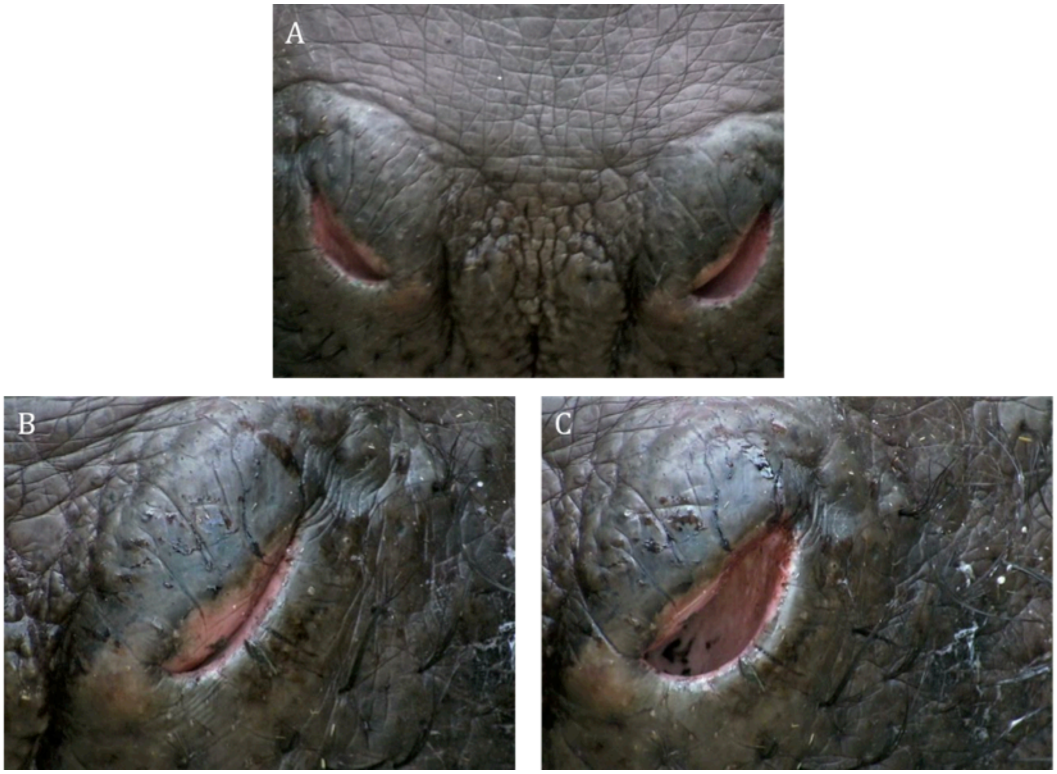


Figure 2.1. States of the nostrils of a male hippo on land: A) partly open, B) closed, C) open.

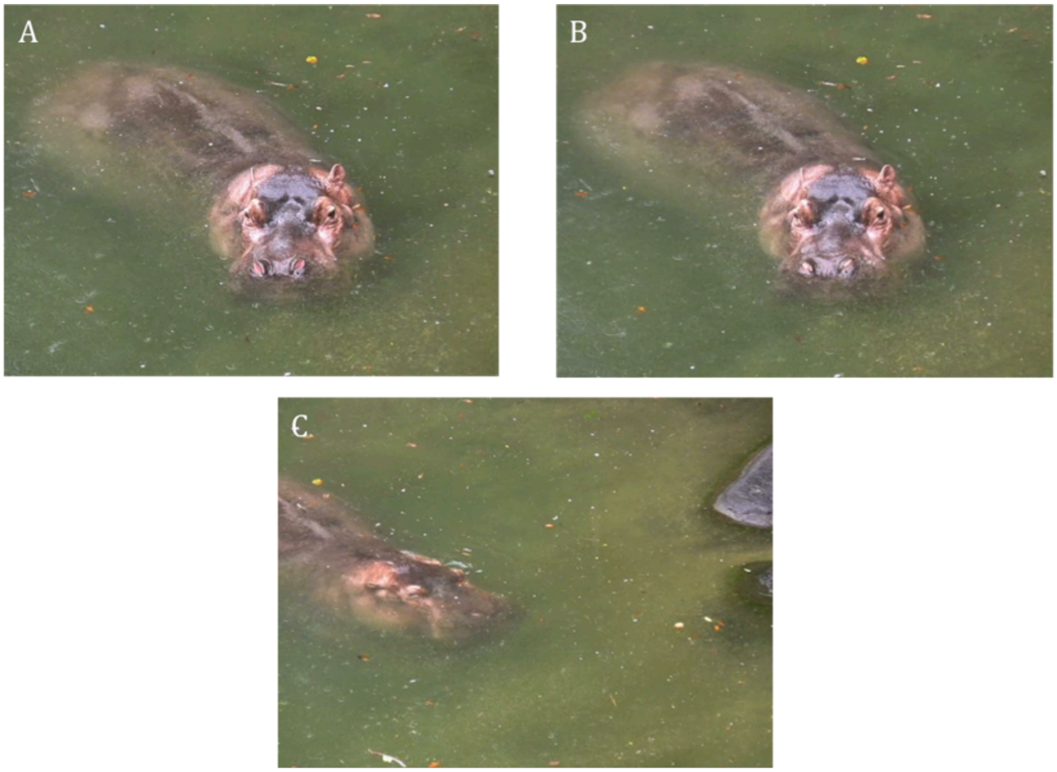


Figure 2.2. States of the nostrils of a male hippo in water: A) open in the amphibious position, B) closed in the amphibious position, C), closed, when submerged.

Discussion

A review of the literature clearly indicates that hippos have several adaptations appropriate for their amphibious lifestyle, as well as some characteristics that support their evolutionary connections to cetaceans. Although there are still some conflicting reports about their adaptations, the structure of their anatomy and sensory systems may represent convergent adaptations to aquatic living and/or shared derived characters from the common ancestors of hippos and cetaceans.

Recent studies suggest that semi-aquatic living pre-dated the hippo-whale clade (e.g., Spaulding et al., 2009). Thus adaptations such as denser limbs for bottom walking, tubular body shape, and the ability to hear underwater using middle ear bones that are partially separated from their skulls may represent inherited characteristics from their common ancestor. It is also possible these characteristics, along with the structures of the hippo eye and valvular nostrils, represent the transition to the fully aquatic state. Their partially webbed feet and unstable gait underwater may represent characteristics that evolved separately or other transitive states. Although respiration rates may be similar to cetaceans, hippos might not demonstrate diving bradycardia, are not capable of staying submerged for very long, and do not swim (Figure 2.3). Further comparisons of these adaptations to aquatic mammals (e.g., Reidenberg, 2007) and additional studies of fossils are needed to resolve these questions.

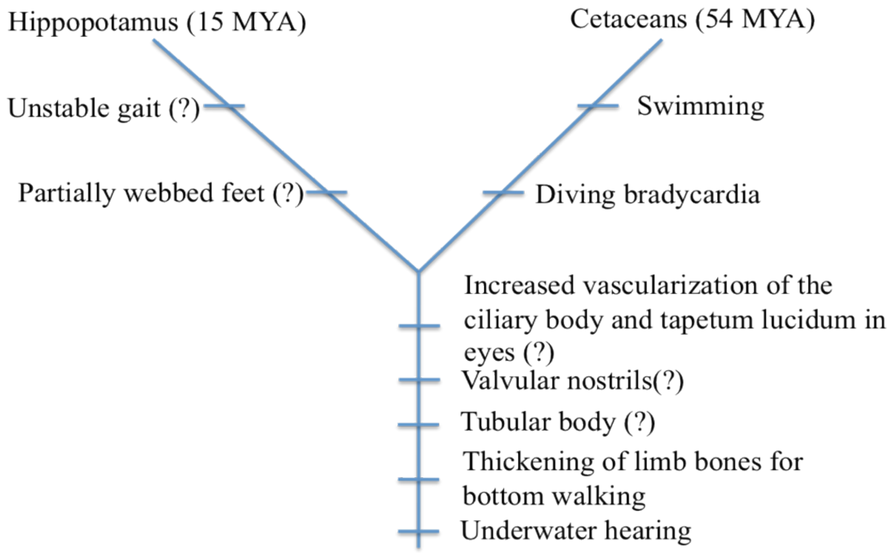


Figure 2.3. Phylogenetic tree representing potential convergent evolution or shared derived characters from the common ancestor of hippos and cetaceans.

The nostrils of the hippo appear to be closed while resting both on land and in the water, suggesting that like cetaceans, they may have control over their breathing. Since hippos primarily rest and sleep in the water, voluntary breathing could represent another aquatic adaptation advantageous for resting in the water. The structures of the muscles of the nostrils are unknown, but visual inspection of the nostrils demonstrated that hippos appear to have control over opening and closing of the nostrils and can also keep them in a half-closed state (Figures 2.1 and 2.2). However, when the hippo was alerted to my presence during these observations, it was not clear whether the nostrils were open due to vigilance or because they were normally open. Further observations of hippos resting in water and on land are needed to confirm the resting state of their nostrils.

Due to the amphibious nature of the hippo and the fact that they can produce sounds separately and simultaneously in two mediums (in-air and underwater), the production, transmission and reception of sounds remain unclear and in need of further investigation. Hippos often remain in the amphibious position, defined by Barklow (1994, 1997, 2004) to represent the position of the head when the eyes, ears, and nostrils are above the surface while the mouth and throat are below. Through observations of captive hippos, it appears that head position (e.g., amphibious position or above the surface) may be the determining factor as to which medium the sounds are transmitted. Very few studies have alluded to amphibious communication in species such as crocodiles and alligators (e.g., Vergne et al., 2009) and harbour seals (Renouf, 1984), but it is unclear whether communication in two media may be a consequence of head or body position or due to the noise levels of certain sounds.

As previously noted, hippos produce acoustic signals with and without emission of bubbles. For several signals, an exhaled mist can be seen when hippos produced sounds, confirming emission through the nostrils. However, it remains unclear as to whether hippos produce sounds from the larynx, nasal system, or both, and further research is needed to determine how signals are produced in-air and underwater.

Further Research

Further studies are needed to investigate both the internal anatomy and capacities of their sensory organs to better understand how hippos have adapted to amphibious living. Dissections of the internal anatomy, especially the nasal cavity and muscles of nostrils will help to determine whether hippos have control over their nostrils (e.g., voluntary breathing) and how they manipulate the nasal muscles for opening and closing. Additional observations and comparisons of the adaptations of hippos and aquatic mammals (e.g., Reidenberg, 2007) are needed to better understand which adaptations may have evolved from the common ancestor of hippos and cetaceans.

Future studies should also investigate other possible sensory systems such as the vibrissae along their mouth, which may be used for foraging or other tactile purposes. In addition, several studies have reported clicks emitted by captive and wild hippos, but these signals have been speculated to be social in function rather than for echolocation (Barklow, 1997). This will be discussed in detail in chapter 5, which includes an experiment to test whether hippos may use click trains for determining the direction and distance of objects, or echo-ranging.

To better understand how hippos communicate, future studies should investigate their vocal organs and pathways for sound production and reception. A complete audiogram through behavioral or electrophysiological tests is needed to determine what frequency range hippos can perceive in relation to the sounds they produce. Additional visual observations of hippos producing sounds and recordings using contact microphones may also be used to localize the source of sounds.

Conclusion

These findings suggest that hippos share a number of adaptations with cetaceans that may represent traits derived from their common ancestor or convergent evolution. Further research is needed to better understand the adaptations of hippos, particularly their sensory systems and mechanisms used for the production and reception of sounds.

Chapter 3. Mechanisms of sound production

Researchers have speculated that hippos produce sounds by passing air from the lungs to the larynx, causing vibrations of the vocal folds (Barklow, 1994, 1997, 2004). Sounds produced by hippos are likely to be emitted through the nostrils, since no bubbles are released underwater from the mouth and sometimes an exhaled mist may be visually observed concurrent with in-air sound production. Underwater sounds do not always include bubbles, suggesting other pathways for sound transmission and propagation. The fold of blubber around the throat may be important for sound propagation (Barklow, 1994, 2004; Feldhake, 2005), but further research is needed to examine the potential role of this structure and other parts of the vocal anatomy of hippos.

Early dissection studies of hippos in the late 19th century provided some of the first descriptions of their anatomy (e.g., Crisp, 1867; Chapman, 1881), including their potential vocal organs. However, these findings were limited because many of these dissections were of fetal or infant hippos (e.g., Crisp, 1867). The larynx was measured to be approximately 6.5 inches long and 6 inches in circumference, and reported to be similar in structure to manatees (Chapman, 1881) and dolphins (Crisp, 1867). Chapman (1881) also noted there was a large, flexible space between the larynx and tongue that can be thrust up to the posterior nostrils, closing off the mouth and allowing airflow to the larynx. This may serve as an advantage for hippos when submerged to allow them to open their mouths underwater. In addition, the external nostrils are flexible, which may enable them to take a deep breath and hold large volumes of air that may be passed through the larynx as needed (Chapman, 1881). Brunton & Cash (1883) noted delayed development of the vocal folds and reported that a hippo fetus had no vocal fold, but rather a "longitudinal elevation by the anterior extremity of the arytenoid." Chapman (1881) described the vocal folds as situated obliquely and measured them to be 2 inches long and 0.5 inches deep.

The pharynx was described as primitive because there was less differentiation of the muscles as compared to other species (Himmelreich, 1981). The pharynx was measured to be 100 mm along the vertebral wall and the width of the aperture of the pharynx to the mouth was 59 mm. In describing the musculature of the pharynx, Himmelreich noted that these features support the ecology of hippos and may be important for feeding on aquatic or riverside vegetation that is shredded during the foraging process (1981). The trachea was described as nearly uniform, with 23 rings, and similar in appearance to dolphins (Crisp, 1867). The nasopharynx was described as a long tube that is shaped like a horn, measuring approximately 16 inches long by 4 inches wide (Barklow, 1994). Himmelreich (1981) also measured the rostroventral and vertebral portions of the nasopharynx at 103 mm and 90 mm. Although these measures have provided some information about the potential vocal organs of hippos, further research is needed to describe the role of the structures during sound production.

Dissection of an adult female hippo

In October 2010, I became aware that colleague Dr. Joy Reidenberg at Mount Sinai Medical School had the opportunity to dissect a hippo for the television show “Inside Nature’s Giants,” which focuses on investigating the internal anatomy of large mammals. I requested that she take specific measurements of the vocal tract and descriptions of the vocal organs of hippos as part of a collaborative effort to better understand their vocal anatomy.

Methods

An adult female hippo was dissected in Zambia in October 2010. The hippo head was not bisected, but measurements were made of the center of the vocal tract from the epiglottis to lips, along with the larynx and vocal folds (Reidenberg, unpublished data, personal communication). To estimate the vocal tract including the nasal cavity, the depth from the nostrils to lips was measured. The tissue on the neck was examined because it was previously hypothesized to serve a role in sound propagation (Barklow, 1994).

Results

The results of the dissection provided information about the position and size of the hippo's anatomy related to sound production that was comparable to previous studies. The vocal tract measured from the vocal folds to the lips was approximately 80 cm and approximately 82 cm to the nostrils. The larynx was approximately 19 cm, and the vocal folds were 7 cm long by 5 cm wide (Table 1). The vocal folds were u-shaped and oriented parallel to airflow (Figure 3.1). The fold on the neck of the hippos was also dissected, but it did not contain fatty tissue, as was expected given its location around the larynx and throat and speculation it may aid in sound propagation.

Table 1. Measures of the internal anatomy of hippos

	Dissection of adult female (October 2010)	**Previous dissection studies**
Pharynx		
Larynx	19.05 cm long	16.51 cm long (Chapman, 1881)
Vocal folds	7.11 cm long, 5.08 cm wide	5.08 cm long, 1.27 cm deep (Chapman 1881)
Vocal tract	80.01 cm to mouth, estimated 81.79 cm to nostrils	
Lungs		3.63 kg (Crisp, 1867)
Mandibles		10.01-60.20 cm (dependent on age) (Laws, 1965)

Figure 3.1. Vocal folds of *Hippopotamus amphibious* (Reidenberg, personal communication) (*denote the shape of the vocal folds).

Discussion

Understanding the exact mechanisms of sound production and transmission is complicated due to the lack of information about the internal structures and physical characteristics of the nasal and oral cavities of hippos. However, data from the dissections of hippos and other related species can provide insights about the possible mechanisms. The recent dissection of the adult female hippo revealed that the vocal folds of hippos are u-shaped and oriented parallel to airflow (Figure 3.1). The vocal folds of hippos are more similar in shape and orientation to baleen whales (Mysticetes), as opposed to most terrestrial mammals where they are perpendicular to airflow (Reidenberg & Laitman, 2007a). Although hippos do not appear to have the laryngeal sac found in baleen whales, the tonal signals they produce suggest they may be capable of producing “voiced” sounds as air passes across the vocal folds (Reidenberg, unpublished data, personal communication).

Exhaled bursts accompany the beginning of wheeze honks, which are tonal signals, suggesting these sounds come from the larynx and are emitted through the nostrils since no bubbles come from the mouth. In addition, a mist of air was visually observed when hippos produced some acoustic signals on the surface, suggesting the nasal system may also be involved in sound production (Feldhake, 2005). One study noted that hippos produced clicks with their mouths open underwater, yet no bubbles were observed (Kreuger, 2000). Clicks produced by hippos are similar in structure to those produced by cetaceans, suggesting that hippos may also produce clicks in the nasal system by shunting air across internal structures in the nasal cavity.

The larynx and vocal folds have been described in most cetaceans, although their role in vocal communication some species is uncertain (e.g., Reidenberg & Laitman, 1987). Most toothed whales generate pulsed signals from the nasal system, which includes pairs of phonic lips (monkey lips-dorsal bursae complex) that sit below the blowhole (Cranford et al., 1996, Cranford, 2000; Reidenberg & Laitman, 2008). Bottlenose dolphins produce clicks by pushing air past the pairs of the phonic lips, causing vibrations of the tissue complex (Cranford et al., 1996). One pulse coincides with the oscillation of the lips as they open and close (Cranford, 2000). Changes in the pulse repetition rate and vibrations of the phonic lips occur simultaneously, suggesting dolphins can produce clicks from both pairs of phonic lips and may have two sound sources (Cranford, 2000). Vibrations of the tissue are projected through the melon area of the dolphin’s head, which contains fatty tissue and aids in directing the sound beam (Cranford et al., 1996; Norris, 1968; Reidenberg & Laitman, 2008).

Previous studies by Barklow (1997) speculated that the fold of blubber around the larynx was important for sound propagation, suggesting it may be similar in structure to the dolphin melon, which contains fatty tissue that helps project and focus high frequency sounds (Norris, 1968). However, the results of the dissection of the female hippo demonstrated that the fold on the neck did not contain any fatty tissue, suggesting it may not be used for sound propagation. Instead, this fold may facilitate movement and positioning of the head, but may differ in structure depending on the sex of the hippo (Reidenberg, unpublished data, personal communication). Further research is needed to confirm this finding to determine the mechanisms of sound transmission and propagation.

I sought to apply source-filter theory to hippo communication, which is based on a two stage model of sound production involving the source (vocal folds in the larynx) and filter (shape and size of the vocal tract). However, due to the orientation of the vocal folds, which are parallel instead of perpendicular to air flow, it was not clear whether the same tube model (open at one end, closed at the other) could be applied to the vocal tract of hippos. I also could not confirm whether the hippos' mouths and/or nostrils were open while producing certain sounds, and it is unclear how their signals were transmitted. Although the tonal calls of hippos suggest laryngeal production and contain formants, further research is needed to determine the location and dispersion of formants to investigate the effects of their vocal tract on the acoustic properties of sounds.

Further research

More research is needed to describe the vocal tract of hippos, particularly through dissections of the oral and nasal cavities of hippos. In addition, source-filter theory can be used to predict the location of formants or vocal tract length based on the acoustic properties of vocal signals (Fitch, 1997). Using the formula $F_n = (2n-1)(c/4L)$, where n is the formant number, c is the speed of sound, and L is the length of the vocal tract, the location of formants can be predicted. For example, Soltis (2010) applied the above principles to investigate the production of rumbles by African elephants. Using knowledge of their vocal anatomy and formant dispersal, the predicted vocal tract length was compared to the actual vocal tract length, which implicated the trunk in rumble production (Soltis, 2010). These principles could be applied to hippo communication to determine whether the mouth and/or nostrils may be involved in sound production and transmission.

Conclusion

The results of this review and dissection provided descriptions of the vocal anatomy of hippos and suggestions for further research for investigating sound production. Their vocal folds were similar in shape and orientation (parallel to airflow) to baleen whales, suggesting hippos may be able to produce sounds as air passes across the vocal folds. Thus, it is possible that hippos produce sounds from the larynx and the nasal system, but further research is needed to better understand the mechanisms of sound production and transmission, particularly for sounds that are simultaneously produced in-air and underwater.

Chapter 4: Acoustic and behavioral repertoires

Little is known about the communication system of hippos, but a few studies suggest that both captive and wild hippos use a variety of acoustic signals that coincide with social interactions (Barklow, 1994, 1997, 2004). Hippos produce a variety of acoustic signals that are transmitted either in-air, underwater, or simultaneously in both mediums, termed “simultaneous amphibious sound” (SAS) (Barklow, 1994, 1997, 2004). SASs are produced simultaneously underwater and in-air while hippos are in the “amphibious position,” with mouth and throat underwater, but nostrils, ears, and eyes above the surface (Barklow 1997, 2004). The speed of sound in water is approximately 1500 meters per second, which is about five times faster than it is in air (340 meters per second) (Au & Hastings, 2008); thus, underwater vocalizations may be especially important for rapid communication between individuals both within the group and in other territories. Playback experiments and observations of hippos demonstrated that they could detect and orient towards underwater and in-air sounds (Barklow, 2004). In Barklow’s study, the hippos were reported to also surface and call in response to some SAS calls, suggesting that the separate components of these sounds are not just a consequence of sound production or transmission.

Acoustic signals

Hippo acoustic signals are generally produced during periods of excitement when they are in the water and most activity tends to occur in the late afternoon, prior to their moving on land to forage (Barklow, 1994, 1997, 2004; Karstad & Hudson, 1986; Walther, 1984). The most common acoustic signal emitted by hippos is the wheeze honk, which is a contact call that is often produced as a chorus by many individuals in the group (Barklow, 1994, 1997, 2004; Estes, 1991; Feldhake, 2005; Karstad & Hudson, 1986). The wheeze honk is produced by males and females in a variety of contexts and is believed to be important for communication within the group and across territories (Barklow, 2004). Hippos also emit a variety of grunts, bellows, and squeals that are very loud and travel long distances (Eltringham, 1999; Karstad & Hudson, 1986), along with exhaled bursts in-air and underwater. When produced in the water, these bursts of air create a fountain-like effect that could be used as a display when hippos are threatened or alarmed (Barklow, 1994, 2004; Karstad & Hudson, 1986). However, it is not clear whether these signals function as communication signals or a result of exertion during social displays.

Barklow (1997) was the first to conduct an in-depth study of wild and captive hippo communication. He described three categories of underwater sounds that included tonal whines, pulsed croaks, and clicks produced when hippos were submerged and with little to no expiration of air. He categorized these signals based on measurements of the acoustic parameters (e.g., duration, fundamental frequency, frequency of peak amplitude, etc.) from spectrograms. Behavioral contexts were included, but were only based on a few observations. He described "tonal whines" which were produced during submissive contexts that may represent more than one category of signals. Pulsed croaks were described as a series of pulses produced as a staccato, which were associated with increased alertness and excitement and were produced during interactions between calves and subadults. Clicks were produced singly or as part of a click train and may include different types of clicks: wideband, narrowband, and inharmonic clicks (clicks with little harmonic content, usually produced as single pulses). Although hippo clicks resemble the echolocation clicks of cetaceans, Barklow (1997) noted these signals were produced during social interactions and believed to coincide with aggressive displays.

In a follow-up study, Barklow (2004) described nine general categories of non-discrete or graded sounds that hippos could produce simultaneously in air and underwater. Hippos spent approximately 77% of their time submerged and most of their acoustic signals were produced underwater (61%), as compared to SAS calls (35%) and signals only heard in air (4%). No signals were recorded that were made exclusively on land. Some of these signals were given alone or as multi-element calls that were referred to as screams, huffs, growls, grunts, tonal calls, clicks and pulsed croaks. The most frequent sounds were underwater clicks, croaks, and whines that were mostly produced without the expiration of air. Hippos also emitted other sounds that included nasal flutters and exhaled bursts of air, but their functions remain unclear (Barklow, 2004).

Barklow (2004) pooled and differentiated hippo acoustic signals by the medium (in-air, underwater, or SAS) in which they were transmitted and did a quantitative analysis based on mean peak frequency, amplitude and sound pressure level (Barklow, 2004). The pooled data had mean peak frequencies below 205 Hz for aerial sounds and below 2087 Hz for underwater sounds. Due to the rapid speed of sound transmission in water, underwater sounds preceded the aerial components of SAS calls, but were lower in intensity as a result of density differences between air and water. In addition, the underwater components of SAS calls had higher mean peak frequencies, which may be due to how these sounds were produced and transmitted. The exhaled bursts of air underwater contained infrasonic components that may be the result of the oscillation of large bubbles (Barklow, 2004).

Behavioral contexts

Although the functions of hippo acoustic signals have been suggested for a few signal types, most remain to be studied. Behavioral contexts are lacking primarily because it is difficult to observe the social interactions of hippos when they are submerged in murky water, which is where a majority of the signals are produced. However, some studies have described the social interactions of hippos at the surface or on land, suggesting that there are important dominance and submissive displays (e.g., Eltringham, 1999; Estes, 1991; Karstad & Hudson, 1986; Klingel, 1991; Olivier & Laurie, 1986). As mentioned above, several hippo sounds have been described to coincide with behavioral displays, such as tonal whines that coincide with submissive contexts (Barklow, 1997) and bubble bursts that may serve as a threat display (Barklow, 1994; Estes, 1991). In particular, wheeze honks are often produced as a chorus that begins when the dominant individual produces the call, suggesting this signal is a contact call important for social cohesion or communication across territories (Barklow, 1994; Estes, 1991; Feldhake, 2005; Walther, 1984).

A recent study demonstrated that social interactions within a captive group of female hippos were influenced by kinship, familiarity, and dominance (Blowers et al., 2009). Although aggressive interactions were infrequent, they included social displays and acoustic signals such as the wheeze honk and snorts that were mostly random, indicating a potential non-linear dominance hierarchy (Blowers et al., 2009). These findings suggest that dominance relationships exist within the group of females, but most aggressive interactions appeared to be random and age and weight did not correlate with rank. Hippo acoustic signals may serve a role in social displays or indicate status, but further research is needed to better understand the functions of these signals.

Research goals

The purpose of this study was to build on previous work by Barklow (1997, 2004) to further describe the acoustic and behavioral repertoires of the common hippo. A better understanding of the functions and mechanisms of hippo acoustic signals can provide more information about social group dynamics and can reveal information about how hippos have adapted to their amphibious lifestyle. Through opportunistic recordings of captive hippos, I sought to describe the acoustic parameters and behavioral contexts of their acoustic signals, as well as sex differences and comparisons of this data to previous work with captive and wild hippos.

Methods

Subjects

The subjects of this study were 16 captive hippopotamuses (*Hippopotamus amphibius)* (7 males, 9 females) ranging from 4-35 years old (at the start of the study) at Disney's Animal Kingdom® (DAK) in Lake Buena Vista, Florida (Table 2). Hippos were separated by sex and housed in two different barns and three separate exhibit pools. The two barns each contained eight stalls (7.6 m long by 4.6 m wide) and one larger stall (7.6 m long by 9.1 m wide). Adjacent to each barn were two holding pools (6.4 m long by 5.2 m wide). Pathways from the barns led to the exhibit pools, which included the river pools (male pool: approximately 70.1 long with variations in width up to 22.9 m wide, depth ranging from 0.5 to 2.7 m; female pool: 106.7 m long by 22.9 m wide, depth ranging from 0.3 to 2.9 m) and underwater viewing pool (walk through area for the public, 19.8 m long and 19.8 m wide, depth ranging from 0.3 to 3.2 m), both of which had variable amounts of shore space around the pool (Figure 4.1).

Table 2. Captive hippos at Disney's Animal Kingdom® (DAK)

(*original group 1998)	**Year of birth**	**Origin (parents)**
Males		
Nacho*	1991	Nueva Leon Zoo, Mexico
Henry*	1995	Leipzig Zoo, Germany
Uzazi	2000	DAK (Norman, Rosie)
Padron	2000	DAK (Norman, Mezcal)
Hans	2001	DAK (Henry, Tuma)
Biko	2002	DAK (Norman, Binti)
Tucker	2003	DAK (Nacho, Tuma)
Females		
Viviana*	1972	Parc Zoologigue de La Palmyre, France
Geraldine	1972	Central Florida Zoo
Rosie*	1990	Naguerdei Kultupark, KHT
Tequila*	1993	Pargue Zoologico de Leon, Mexico
Mezcal*	1993	Parque Zoologico de Leon, Mexico
Tuma*	1996	Zoologischer Garten, Basel
Binti	1998	Denver Zoo
Ramona*	Unknown	Hermosillo, Mexico
Panchita*	Unknown	Francisca Centro Ecologico de Sonora, Mexico (Nacho, Ramona)

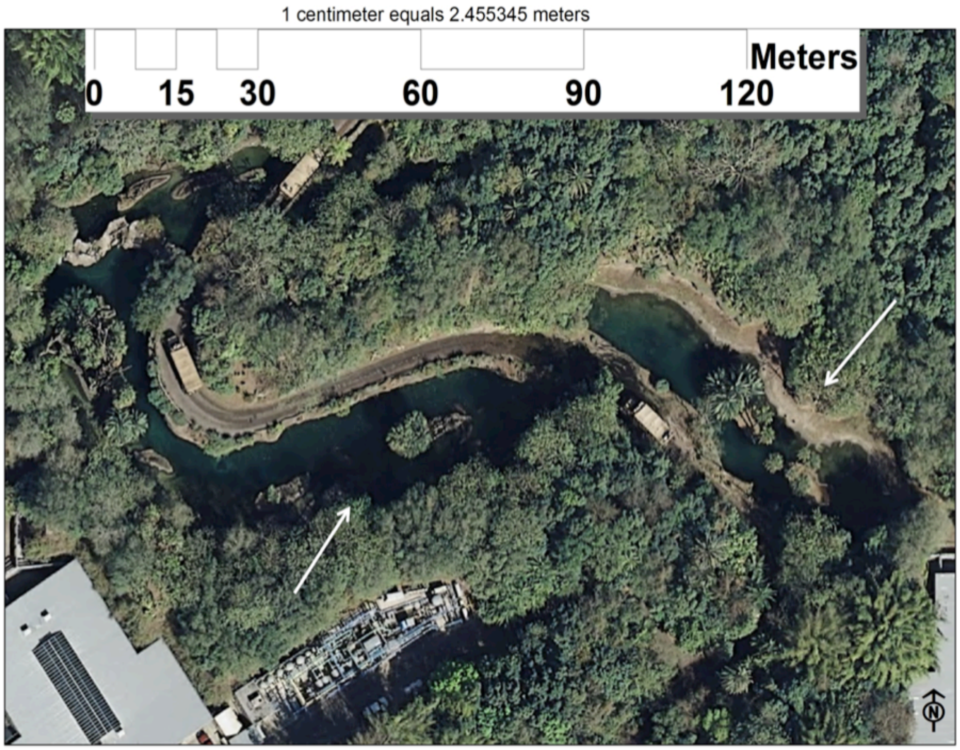


Figure 4.1. Configuration of the hippo river pools. The male pool is denoted by the arrow on the right and the female pool is noted on the left.

Hippos were rotated daily to different stalls and shifted between the different holding pools and exhibit pools. Hippos were given approximately 25% of their daily portions of hay and

50% of their daily produce and grain before going out into the pools. Hippos were also given food enrichment throughout the day both on land and in the pools. The group of nine females generally spent each day in the river pool and were called in to the barns around dusk were they remained for the night. The male hippos were generally housed alone except for some of the younger males (Hans, Biko, Tucker) that could share pools with other males. Henry and Hans (father and son) were frequently together in the river pool, as well as Biko and Tucker until Tucker was sent to another facility in 2009. The males had a rotating schedule that determined which hippos were out in the exhibit pools (river or underwater viewing) or holding pools during the day or at night.

Recording methods

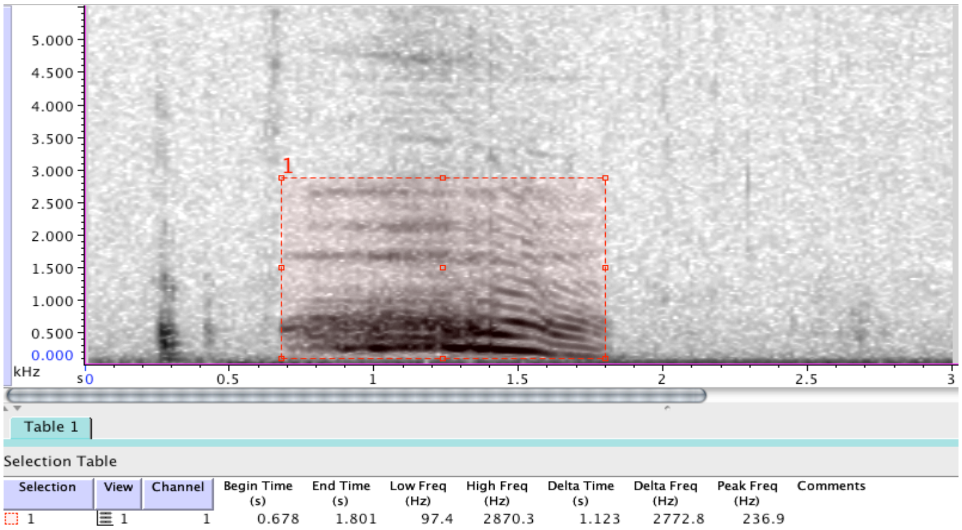
Simultaneous video and audio recordings were collected during summer months (one week in August 2007, June-August 2008, and July-August 2009), using a Vixia HV20 Canon digital video camera, C54XRS Cetacean Research Technology Hydrophone (0.008-310kHz (+1/-20dB)), QTC 50 Earthworks microphone (0.003-50kHz (+1.5dB)) and HD-P2 Tascam digital audio recorder (0.02-20kHz (-1.0dB); 44.1 kHz sample rate). Opportunistic recordings were collected from male and female hippos in their exhibit pools six days a week (Monday-Saturday) between the hours of 5:30 and 8:30 A.M., prior to the facility opening to the public and when the pool filters were turned off. A few recordings were also made in other contexts: holding pools (n = 3 sessions (one male, two females)), male barn at night (n = 1 session), the male river pool at night (n = 1 session), and when female hippos were coming into the barn in the evening (n = 5 sessions).

A total of 146 sessions, approximately one hour in duration (ranging from 5 to 109 minutes, depending on park opening and the goal of the session) were conducted to record the ambient noise, acoustic signals, and concurrent behavior of hippos. Experimental sessions with five males (n = 68 sessions) and pseudo-experimental sessions with the social group of females (n = 8 sessions) when carrots were offered were included in this analysis (*discussed in Chapter 5*). After the completion of the experimental trials with the males, recordings were continued to observe potential interactions.

Analysis

Acoustic signals

Audio recordings were analyzed separately from the video files to determine the occurrence of different acoustic signals. The mean number of signals per session was used to compare the frequency of use of signals adjusted for variations in the length of the session. Spectrograms were generated in Raven acoustical analysis software (version 1.3) (Spectrogram parameters: Hanning window size = 1586 samples, 3db filter bandwidth = 40 Hz, overlap = 90%, frequency grid DFT size = 2048 samples) and examined visually to draw a box around each signal to measure the acoustic parameters (Figure 4.2). The following acoustic parameters were measured and calculated in Raven: minimum (lower frequency bound (Hz)) and maximum (upper frequency bound (Hz)) frequencies, bandwidth (difference between the minimum and maximum frequency (Hz)), duration (onset to the end of the signal (seconds)), and peak frequency (frequency (Hz) with highest amplitude). Amplitude was not measured because the distance or orientation of the moving hippos to the hydrophone could not be determined.



Selection Table

Selection	View	Channel	Begin Time (s)	End Time (s)	Low Freq (Hz)	High Freq (Hz)	Delta Time (s)	Delta Freq (Hz)	Peak Freq (Hz)	Comments
1	1	1	0.678	1.801	97.4	2870.3	1.123	2772.8	236.9	

Figure 4.2. Sample of “boxed” signal selection in Raven and measures in the selection table.

Categorization of signals

One approach to categorizing the signals was to compare to their acoustic parameters. First, the parameters of sounds were plotted (e.g., peak frequency versus bandwidth) in Excel (Microsoft Office 12.3.0) to look for possible clusters of signal types. Second, visual and aural inspection of the spectrograms was also used to identify the signal categories (bursts of air, tonal, and pulsed). The acoustic parameters of signals from each category were measured. Signals were categorized by signal type and medium (air versus water) in which they were produced. Autocorrelations of the signals in each category in PASW (version 18.0) demonstrated that the data were not independent, which prevented comparisons of the mean parameters of the signals.

To describe the different signal types (chuffs, snorts, bubble displays, groans, grunts, screams, whines, wheeze honks, clicks, croaks, and growls) within the categories (burst of air, tonal, pulsed), ten signals were randomly selected from male and female hippos of each possible subcategory in the medium in which they were primarily produced. In addition to the acoustic parameters mentioned above, other measurements were made to describe and quantify the physical characteristics of the signals in the different categories. Specifically, for the burst of air category, chuffs and snorts were analyzed using the same measures listed above. Although chuffs often occurred as a bout of 1 to 8 signals, each chuff was analyzed separately within a bout. For the tonal category, measures of the fundamental frequency (F_0, lowest frequency of the signal) were included for groans, grunts, screams, and whines. Wheeze honks were part of the tonal category, but they were analyzed separately and broken up into the wheeze component and honks (ranging from 2 to 9) for males and females. For each component, the bandwidth and the fundamental frequency were measured. Since wheeze honks were most often produced as SASs and as a chorus, the in-air and underwater components were examined separately. For the pulsed category, measures of the interclick interval (ICI) and individual pulses were included to compare croaks, clicks and growls (Figure 4.3). Principle Components Analysis (PCA) (Varimax rotation) and leave-one-out cross validation (stepwise function) were used to test whether these acoustic parameters could be used to differentiate the signal types within the categories using PASW (version 18.0). The randomly sampled signals were included in separate tests for each category (burst of air, tonal and pulsed) to examine the factor loadings of the PCA and the percent correctly classified in the predicted categories from the leave-one-out cross validation.

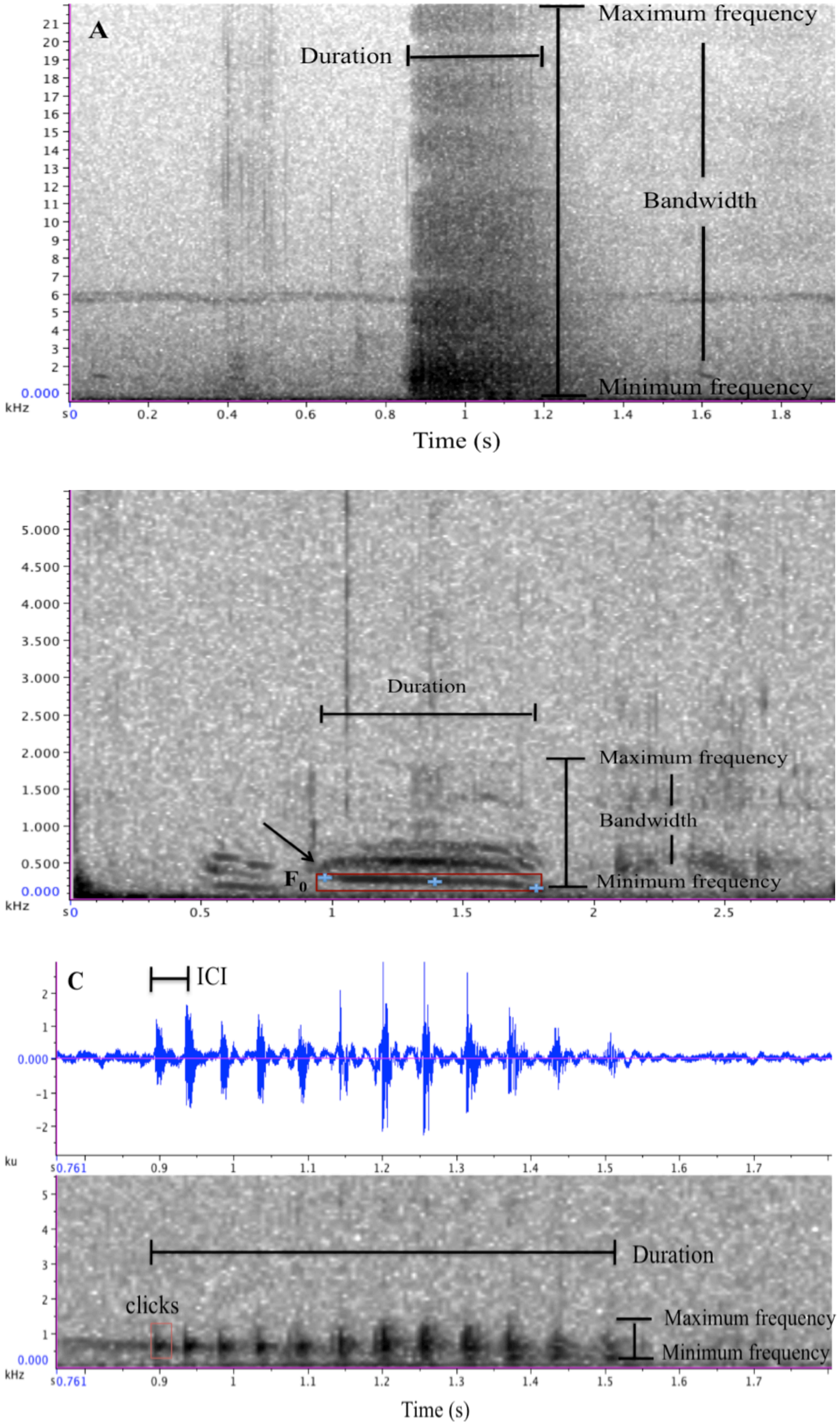


Figure 4.3. Measures of signals in the three categories: A) burst of air, B) tonal, C) pulsed (ICI = interclick interval) (note: peak frequency was calculated in Raven).

Behavioral contexts

Videotapes were analyzed in Subtrak (version 2.1) to observe interactions between individuals in the social group to determine the behavioral contexts of the acoustic signals. These analyses focused on the females since the males were involved in the experimental trials and were rarely housed with another individual. However, the male tapes were included to observe potential interactions following the experiments. Many interactions occurred below the surface and in murky water, so this analysis focused on surface interactions and interactions when the hippos were visible. Each tape was analyzed to note interactions within the group with specific descriptions of the interactions between individuals (*See Appendix for descriptions of behaviors*).

To identify the behavioral contexts, the video and audio recordings were synchronized and the occurrence of behavioral interactions was matched in time. Twenty behavior files (n = 5 videos of males, n = 15 videos of females) were randomly sampled to determine whether there were acoustic signals produced within a 30-sec time window in relation to specific behaviors to determine the behavioral contexts of the signals. Due to the limitations of the viewing window of the camera and murky conditions of the water, hippos were often out of sight and thus the type of interaction could not always be determined.

Results

A total of 1532 acoustic signals were collected from male and female hippos during 124 sessions. During 22 sessions, no acoustic signals were recorded. Ninety-four bubble displays were produced by males and females as a SAS signal. However, bubble displays were excluded from the analysis of the parameters because the acoustic parameters were difficult to measure. Out of the remaining 1438 signals, the group of nine females produced a majority of the signals (80% across 59 sessions), as compared to the males (either alone or in pairs) (20% across 87 sessions). Both males and females produced sounds separately in-air (females n = 179 (mean 3.03 signals), males n = 162 (mean = 1.86 signals)) and underwater (females n = 788 (mean = 13.35 signals), males n = 101 (mean = 1.16 signals)) or in the SAS condition (female n = 189 (mean = 3.20 signals), males n = 18 (mean = 0.20 signals)). Most of the acoustic signals were produced solely underwater (61%) (Figure 4.4).

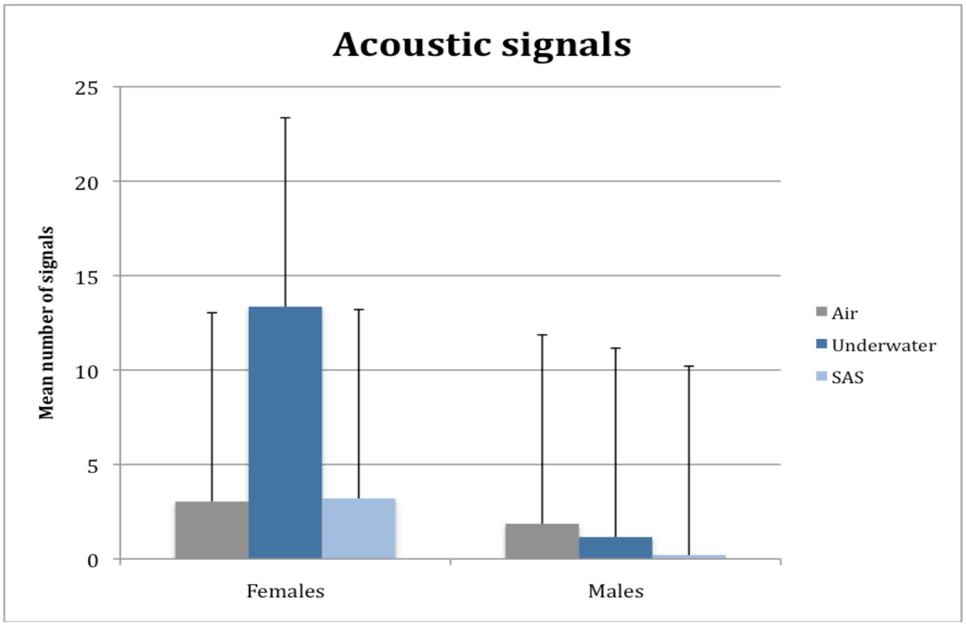


Figure 4.4. Mean number of signals produced by females (n = 9 hippos, 59 sessions) and males (n = 1 to 3 hippos, 87 sessions) produced in-air, underwater, or SAS.

Using the broad acoustic parameters of minimum and maximum frequency, duration, bandwidth and peak frequency, Figure 4.5 is plot of peak frequency against bandwidth for individual signals (in-air and underwater). The signals appear to fall into two general categories of signals that were broadband in frequency (with higher frequency components) or narrowband in frequency (with lower frequency components).

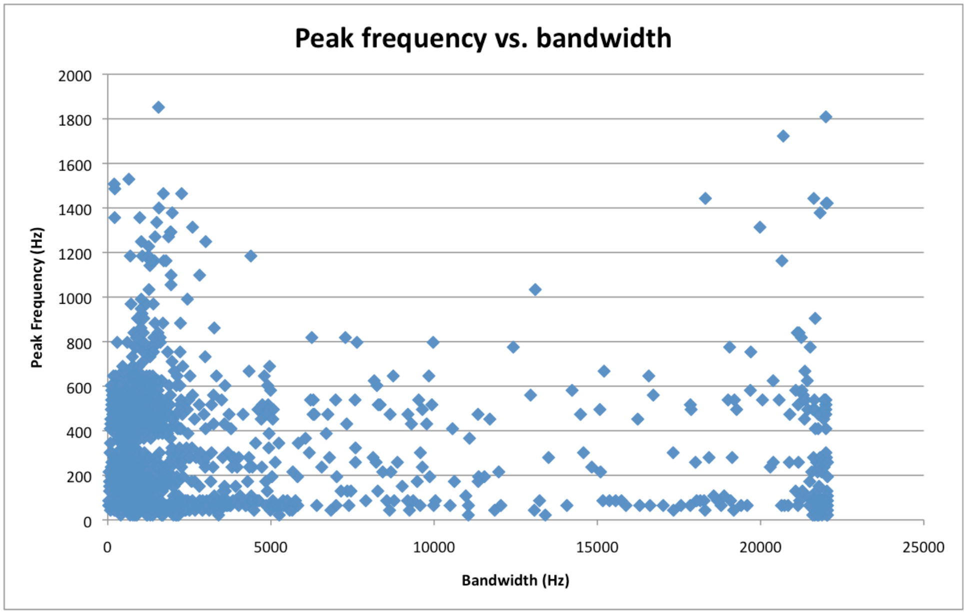


Figure 4.5. Scatter plot of all signals using peak frequency versus bandwidth (Hz).

Visual inspection of spectrograms and aural review of these sounds led to the categorization of acoustic signals into three general signal types: bursts of air, tonal, and pulsed signals. These terms were adapted from the previous descriptions of similar categories of sounds described by Barklow (1997, 2004). Both males and females produced all signal types separately in-air and underwater (burst of air: females n = 59 (mean in-air = 1 signal), males n = 31 (mean in-air = 0.36 signals); tonal: females n = 645 (mean in-air = 1.83 signals, mean underwater = 9.10 signals), males n = 173 (mean in-air = 1.52 signals, mean underwater = 0.47 signals); pulsed: females n = 263 (mean in-air = 0.20 signals, mean underwater = 4.25 signals), males n = 60 (mean underwater = 0.69 signals)) or as an SAS signal (burst of air: females n = 6 (mean = 0.10 signals) , males n = 3 (mean = 0.03 signals); tonal: females n = 133 (mean = 2.25 signals), males n = 13 (mean = 0.15 signals); pulsed: females n = 50 (mean = 0.85 signals), males n = 2 (mean = 0.02 signals)) (Figure 4.6). Tonal signals were the most frequent signal type (67%) for both males and females.

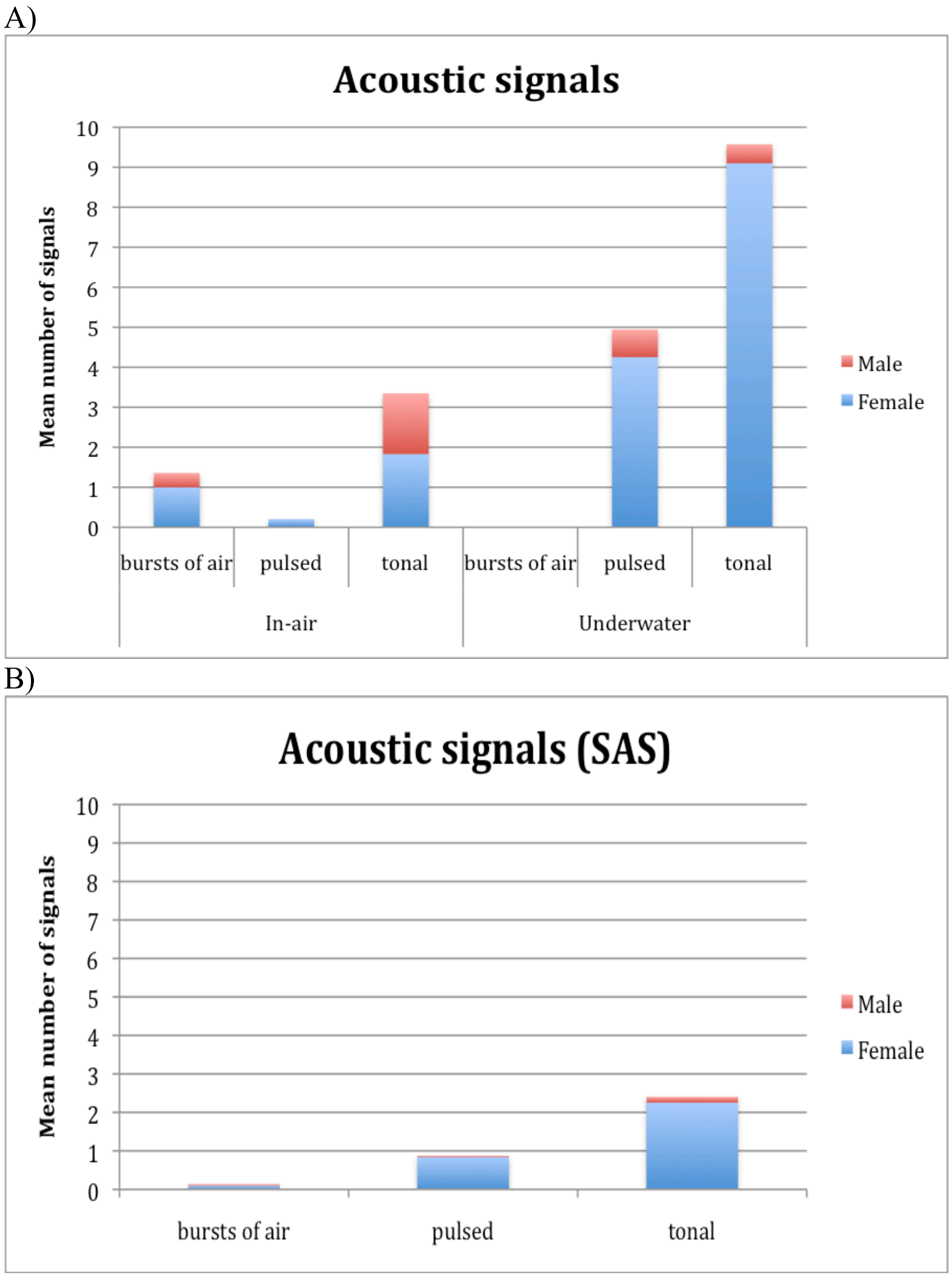


Figure 4.6. Mean number of signals produced by males (n = 1 to 3 hippos, 87 sessions) and females (n = 9 hippos, 59 sessions) by category for A) signals produced separately in-air or underwater, and B) SAS signals (excluding bubble displays).

Burst of air signals

Bursts of air signals were noisy, exhaled bursts that were emitted in-air through the nostrils (based on visual observation). Two signals types, chuffs and snorts, were distinguished by the mean values for minimum frequency, duration, and peak frequency (Table 3). Chuffs were produced as a bout of repeated (1 to 8), short exhaled bursts that were higher in frequency (mean minimum frequency = 1503 Hz, mean peak frequency = 2824 Hz) and shorter in duration (mean = 0. 297 s) than snorts (mean minimum frequency = 100 Hz, mean peak frequency = 288 Hz, mean duration = 0.395 s) produced as single exhaled bursts. Chuffs were the only signals produced solely in-air, while snorts and bubble displays could be produced as SAS signals. Although I did not include the measurements of the bubble displays, they often contained infrasonic components (Figure 4.7).

Table 3. Mean parameters and standard deviations for the burst of air signal types produced by male and female hippos

	Minimum Frequency (Hz)	**Maximum Frequency (Hz)**	**Bandwidth (Hz)**	**Duration (s)**	**Peak frequency (Hz)**	**Bout**
Chuff (n = 10 bouts, 37 total)	1504±1264	20519±2825	19015±2695	0.297±0.091	2825±2334	0-8 chuffs
Snort (n = 10)	100±52	20828±2725	20723±2718	0.295±0.112	228±339	None

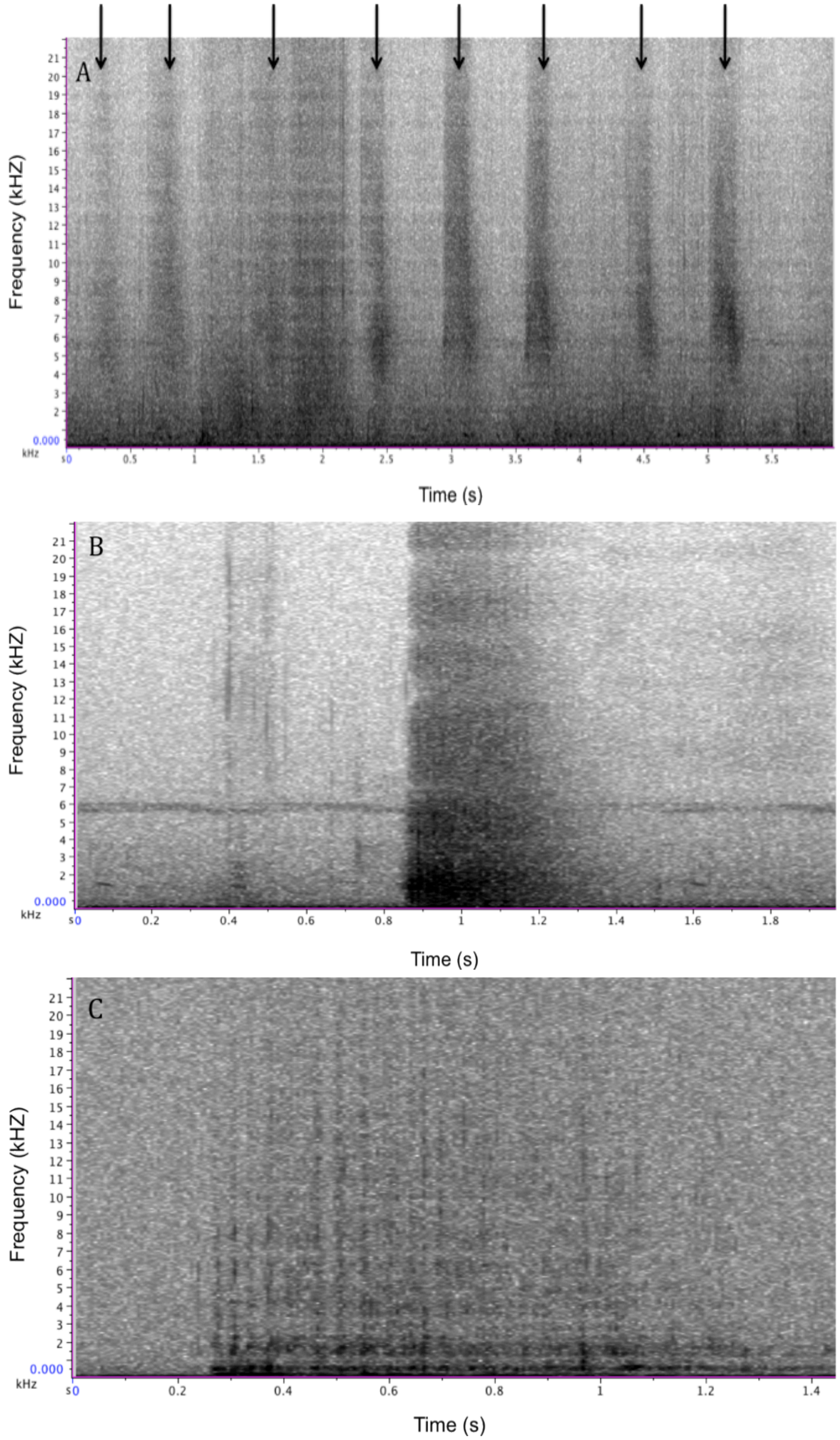


Figure 4.7. Spectrograms of burst of air signals produced by male and female hippos: A) chuffs (arrows denote the 8 chuffs in this bout), B) snort, C) bubble display.

The results of the PCA demonstrated two components (Eigen values >1) that accounted for 85% of the variance in this category. The first component included the minimum and peak frequency representing chuffs. The second component included maximum frequency, bandwidth and duration, representing snorts (Table 4). The results of the leave-one-out cross validation correctly classified the signals by type at 80.9%. FIGURE 4.8 is a plot of frequency (Hz) over minimum frequency (Hz) with clusters representing chuffs and snorts based on bandwidth and peak frequency.

Table 4. PCA for burst of air signals (Varimax rotation)

	Component	
	1 (chuffs)	2 (snorts)
Minimum frequency	0.971	0.039
Maximum frequency	0.160	0.955
Bandwidth	-0.282	0.942
Duration	-0.358	-0.556
Peak frequency	0.970	0.054

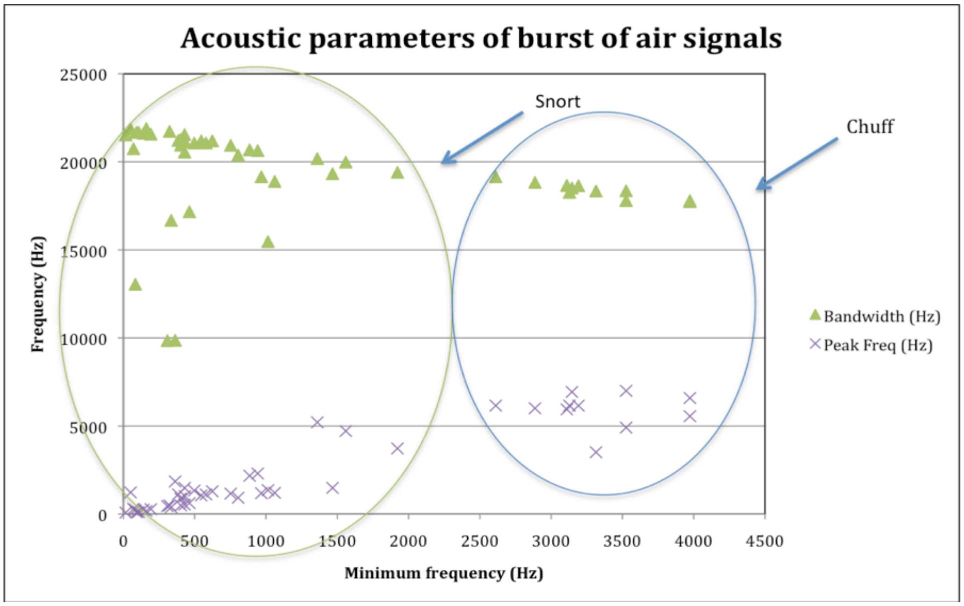


Figure 4.8. Acoustic parameters for the burst of air signals with clusters representing chuffs and snorts based on bandwidth and peak frequency.

Tonal signals

Tonal signals were produced as single calls, longer in duration than other signal types that were visually identified as having energy at a narrowband of frequencies, sometimes with harmonics (Table 5, Figure 4.9). Groans and whines were produced only underwater, screams were produced in-air, and grunts were produced as SAS signals. On a few occasions, groans and screams could be detected as SASs, but this may have been due to the intensity of the sound. Whines and screams were relatively flat signals, while grunts generally started and ended at lower frequencies, but were higher in frequency in the middle. Groans were flat at the first part of the signal and decreased in frequency at the end (Figure 4.9). The minimum frequency (mean = 321 Hz), peak frequency (mean = 549 Hz), and fundamental frequency (mean = 413 Hz) of whines were higher than the other signals. Groans had the highest maximum frequency (mean = 2080 Hz), and grunts had the shortest duration (mean = 0.317 Hz) (Table 5).

Table 5. Mean parameters and standard deviations for the tonal signal types produced by male and female hippos

	Minimum Frequency (Hz)	**Maximum Frequency (Hz)**	**Bandwidth (Hz)**	**Duration (s)**	**Peak frequency (Hz)**	**F_0 (Hz)**
Groan (n = 10 underwater)	125±60	2080±2079	1955±2096	0.613±0.291	314±235	220±99
Grunt (SAS n = 10)	112±53	1298±907	1186±867	0.317±0.206	310±280	146±59
Scream (SAS n = 10)	182±46	824±518	641±509	0.735±0.439	269±119	256±123
Whine (n =10 underwater)	321±100	1093±616	772±635	0.731±0.614	549±289	413±73
Wheeze (air)	121±88	6087±1543	5966±1566	0.442±0.111	200±100	166±93
Wheeze (underwater)	162±130	7022±6193	6860±6251	0.403±0.098	436±170	209±163
Honks (n = 50 air)	47±10	4343±1444	4296±1448	0.167±0.039	75.78±11	76±11
Honks (n = 42 underwater)	118±136	5719±3658	5602±3709	0.214±0.217	359±200	143±109

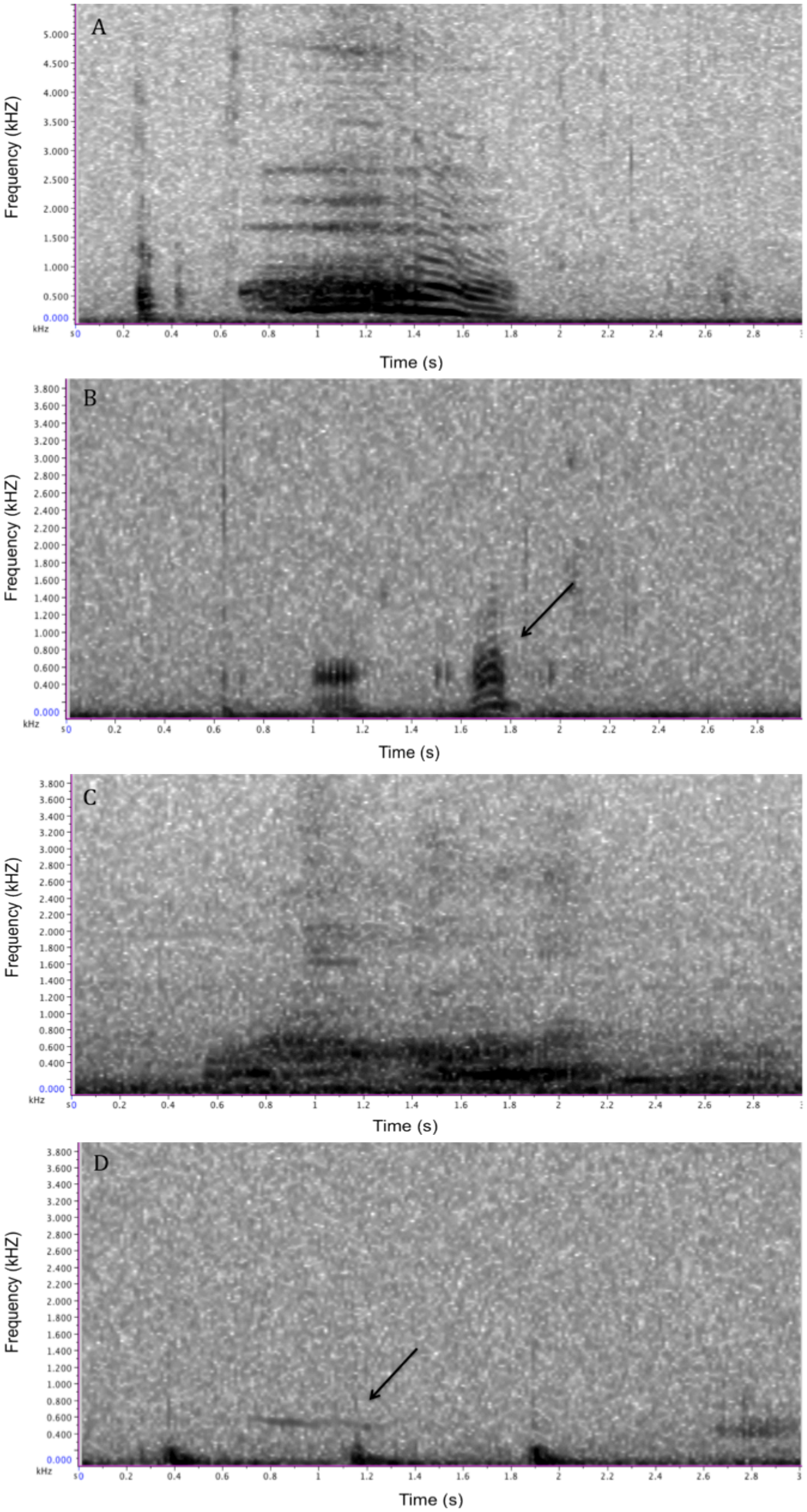


Figure 4.9. Spectrograms of tonal signals (underwater) produced by male and female hippos: A) groan, B) grunt, C) scream, D) whine (arrows denote the signal when other sounds are also on the spectrograms).

The results of the PCA demonstrated 3 components (Eigen values >1) that accounted for 90% of the variance in this category. The first component included minimum, peak frequency, and F_0, representing whines. The second component included maximum frequency and bandwidth, representing groans. The third component included duration, representing grunts (Table 6). The results of the leave-one-out cross validation correctly classified the signals by type at 57.5%. Figure 4.10 is a plot of frequency over minimum frequency demonstrating clusters representing groans, grunt, and whines based on bandwidth, peak frequency, and fundamental frequency (F_0).

Table 6. PCA results for tonal signals (Varimax rotation)

	Component		
	1 (whine)	2 (groan)	3(grunt)
Minimum frequency	0.872	-0.186	0.261
Maximum frequency	-0.009	0.995	0.021
Bandwidth	-0.078	0.994	0.000
Duration	0.117	0.040	0.949
Peak frequency	0.792	0.158	-0.268
F_0	0.912	-0.099	0.250

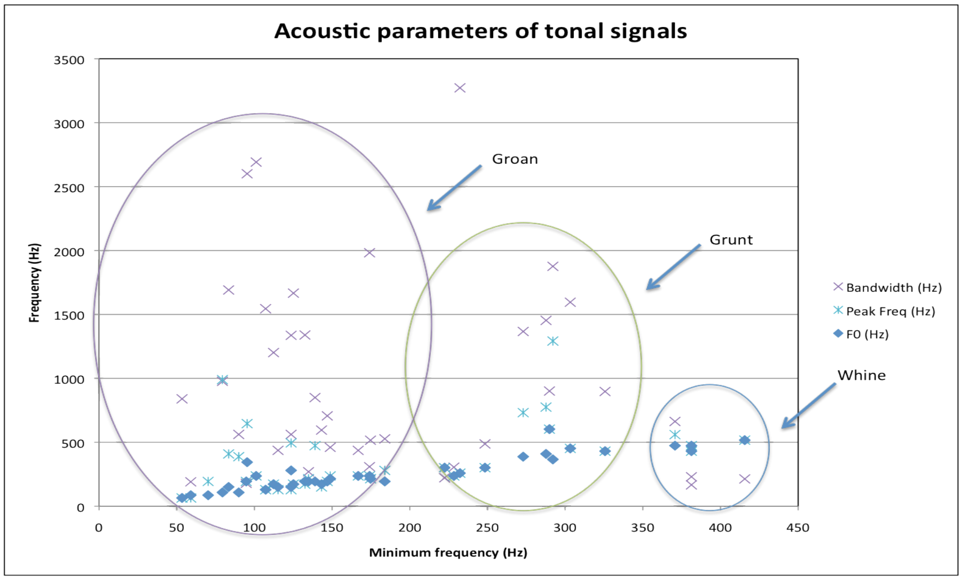


Figure 4.10. Acoustic parameters of tonal signals with clusters representing groans, grunts, and whines based on bandwidth, peak frequency, and fundamental frequency.

Wheeze honks were tonal signals, but analyzed separately because they were often produced as a chorus with overlapping calls produced by several individuals and as SAS signals. The in-air and underwater components of wheezes and honks were analyzed separately for calls in the amphibious position (Figure 4.11). Both the wheeze and honk components appear to be similar above and below the surface, although the in-air component of the wheeze had a lower peak frequency (mean = 200 Hz) than the underwater component (mean = 436 Hz) (Table 5). For the honks, the in-air component had lower frequency measures for the minimum frequency (mean = 47 Hz), maximum frequency (mean = 4343 Hz), peak frequency (mean = 75.78 Hz), and fundamental frequency (mean = 76 Hz) (Table 5).

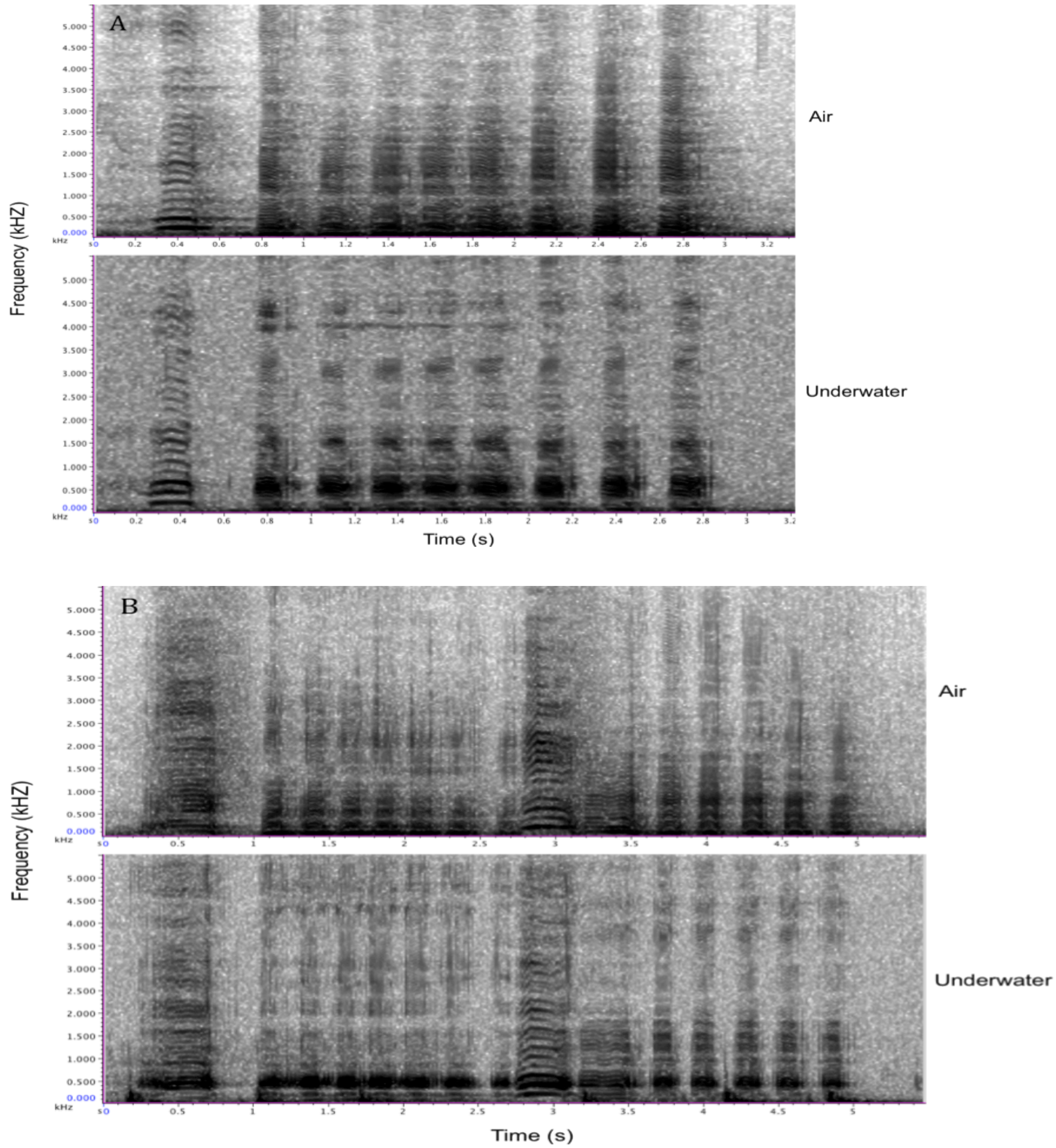


Figure 4.11. Spectrograms of wheeze honks (SAS): A) male, B) female chorus (at least two individuals).

The results of the PCA for the wheezes demonstrated 2 components (Eigen values >1) accounting for 78% of variance. The first component included maximum frequency, bandwidth and duration, representing the underwater wheezes. The second component included the minimum frequency, peak frequency, and $F_{0,}$ representing in-air wheezes (Table 7). The results of the PCA for the honks demonstrated 3 components (Eigen values >1) that accounted for 95%

of the variance. The first component included maximum and bandwidth, representing the underwater honks. The second component included minimum and fundamental frequency, representing the in-air honks. Peak frequency loaded almost equally onto the first and second components. The third component included duration, which may have been due to the longer duration of choruses (Table 7). The results of the leave-one-out cross validation correctly classified the wheezes at 86.4% and honks at 87% based on whether they were produced in-air or underwater.

Table 7. PCA for wheeze and honks (Varimax rotation)

	Wheeze		Honks		
	Component		Component		
	1 (in-air)	2 (underwater)	1 (in-air)	2 (underwater)	3
Minimum frequency	-0.330	0.904	-0.105	0.984	0.004
Maximum frequency	0.962	-0.0.85	0.976	-0.144	0.006
Bandwidth	0.962	-0.107	0.972	-0.177	0.006
Duration	0.481	-0.069	-0.008	-0.008	0.999
Peak frequency	0.337	0.789	0.693	0.600	-0.075
F_0	-0.369	0.889	0-.134	0.976	-0.005

When males and females were compared, female wheezes (mean minimum frequency = 89 Hz, mean F_0 = 129 Hz) and honks (mean F_0 = 82 Hz) were lower in frequency than the males (Table 8). The results of the leave-on-out cross validation supported the classification of wheezes at 81.8% and honks at 62% based on males versus females.

Table 8. Comparison of female and male wheeze honks

	Minimum Frequency (Hz)	**Maximum Frequency (Hz)**	**Bandwidth (Hz)**	**Duration (s)**	**Peak frequency (Hz)**	**F_0 (Hz)**
Wheeze (female)	89±49	7316±5170	7227±5173	0.421±0.089	263±169	129±61
Wheeze (male)	234±129	5221±2458	4988±2574	0.425±0.133	414±167	291±161
Honks (female)	60±51	5135±2867	5075±2846	0.171±0.030	195±177	82±51
Honks (male)	97±125	4822±2682	4725±2747	0.204±0.206	214±212	130±140

Pulsed signals

Pulsed signals were identified as sequences of three or more pulses (ranging from 3 to 56 pulses). Each pulse was less than 0.03 seconds long, with little to no change in the interclick intervals (ICI) within the train of pulses (Table 9). Clicks and croaks were produced only underwater, and growls were produced as SAS signals (Figure 4.12). Croaks were shorter in duration (mean duration = 0.245 s) than either clicks (mean duration = 0.545 s) or growls (mean duration = 0.517 s). The ICI of click trains was longer (mean = 0.048 s) than croaks (mean ICI = 0.034 s) or growls (mean ICI = 0.032 s). Growls had the lowest maximum frequency (mean = 1081 Hz) (Table 9).

Table 9. Mean parameters and standard deviation for the pulsed signal types produced by male and female hippos

	Minimum Frequency (Hz)	**Maximum Frequency (Hz)**	**Click duration (s)**	**Peak frequency (Hz)**	**Duration (s)**	**ICI**
Clicks (n = 10 underwater)	284±93	2279±1496	0.022±0.007	647±302	0.545±0.261	0.048±0.013
Croak (n = 10 underwater)	224±65	2722±2606	0.020±0.005	505±182	0.245±0.083	0.034±0.008
Growl (SAS n = 10)	292±134	1081±660	0.017±0.004	475±167	0.517±0.245	0.032±0.009

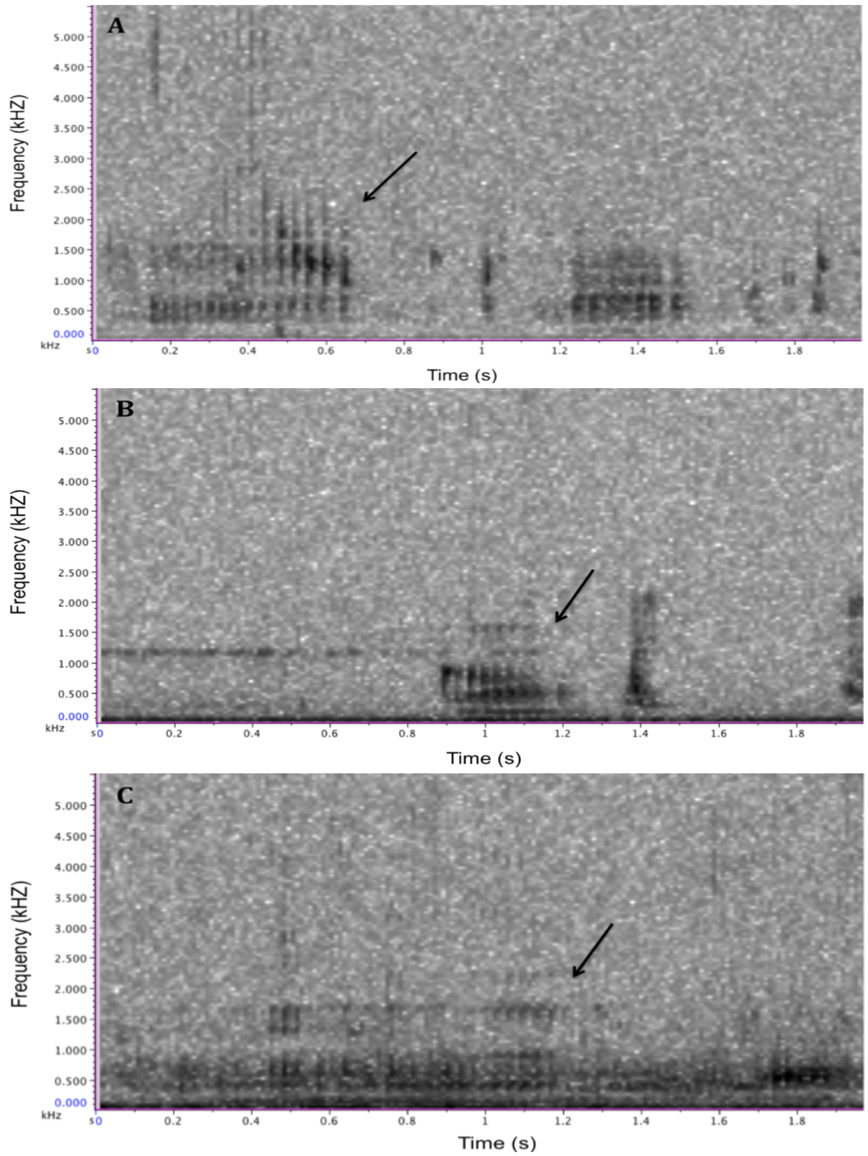


Figure 4.12. Spectrograms of pulsed signals (underwater) produced by male and female hippos: A) clicks, B) croak, C) growl (arrows denote the signals when other noise was present).

The results of the PCA demonstrated three components (Eigen values >1) accounting for 78% of variance. The first component included click duration, and ICI, representing click trains. The second component included minimum and peak frequency, representing croaks. The third component included maximum frequency and duration, representing growls (Table 10). The results of the leave-one-out cross validation correctly classified the signals by type at 68.8%. Figure 4.13 contains plots with clusters representing clicks, croaks, and growls based on maximum frequency and peak frequency (frequency (Hz) over minimum frequency (Hz)) and duration of the pulses and duration of the signals (time (s) over ICI (s)).

Table 10. PCA results for pulsed signals (Varimax rotation)

	Component		
	1 (click train)	2 (croak)	3 (growl)
Minimum frequency	0.063	0.861	0.095
Maximum frequency	0.191	0.373	-0.652
Click duration	0.943	0.044	0.017
Peak frequency	0.009	0.918	-0.169
Duration	0.180	0.155	0.791
ICI	0.941	0.045	0.032

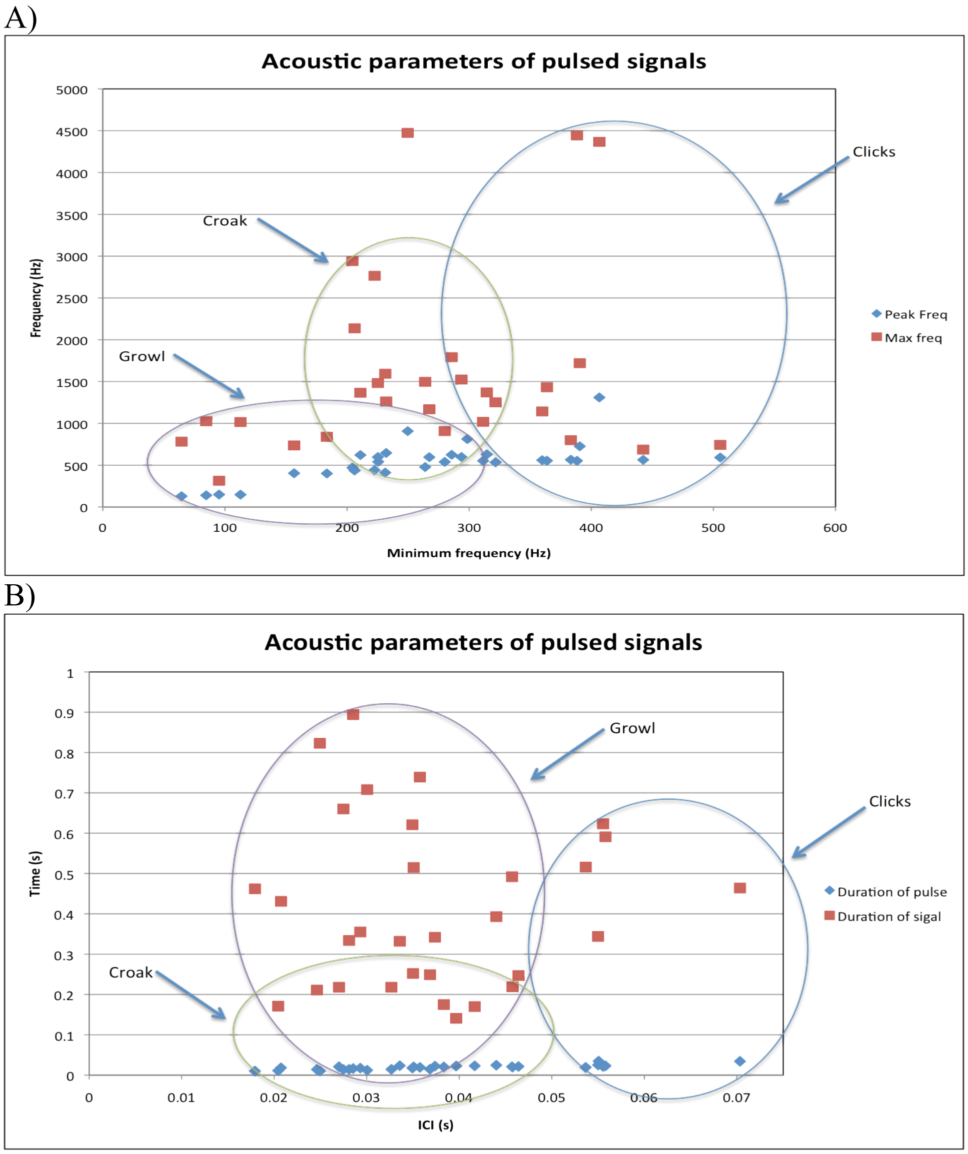


Figure 4.13. Acoustic parameters of pulsed signals with clusters representing clicks, croaks, and growls based on: A) maximum and peak frequency, B) duration of pulses and signals.

Behavioral contexts

Although the acoustic signals appeared to be associated with behavioral interactions, groans, whines, croaks and clicks were produced underwater, which made it difficult to determine their behavioral contexts. In addition, screams were often produced when the female hippos were coming out of the barns and were out of sight. Other occurrences of screams were observed during agonistic interactions, and staff confirmed the contexts of the screams when hippos were out of sight. Two main types of social interactions were identified that involved behaviors previously reported to involve submissive (or appeasement) or dominance (low or high-levels of aggression) contexts (Figures 4.14 to 4.16) (*See Appendix for ethogram*).

Figure 4.14. Examples of submission or appeasement: A) tail paddle, present rear to dominant individual, B) rafting, dominant individual rests head on submissive individual.

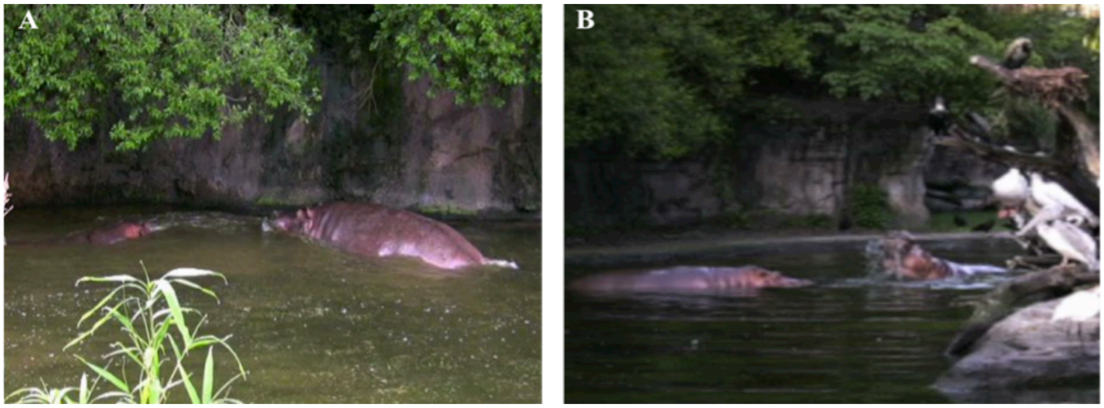


Figure 4.15. Examples of low-level aggression: A) approach, dominant individual arches neck and moves toward other individual, B) head slap, dominant individual lifts head above the surface and drops it down to make a splash on the surface.

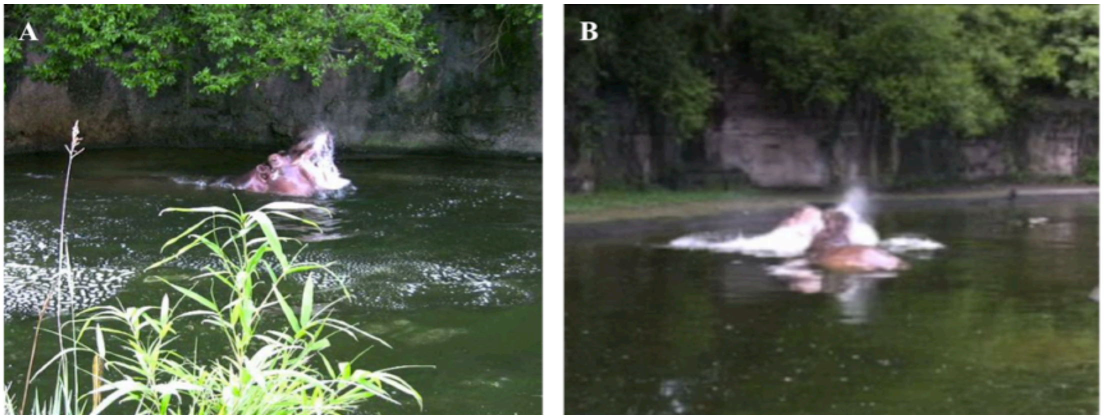


Figure 4.16. Examples of high-level of aggression: A) open mouth with a charge, B) jaw clashing.

In the burst of air category, snorts were associated with dominance interactions and were produced after interactions or when one hippo was approaching another individual (n = 4 occurrences). Chuffs were associated with submissive contexts observed when one male produced chuffs in response to the approach of the more dominant male as he entered the pool. Females also produced chuffs as they moved away from the approaching or charging individual (n = 1 occurrence) or following low-level aggression such as a dominant individual displaying an open mouth scoop or head thrusting (n = 7 occurrences) (Table 11).

In the tonal category, grunts were associated with dominance contexts since they were produced prior to, during, and following low-level aggression (n = 19 occurrences) and high-level aggression (n = 2 occurrences) such as open mouth scoops, approaches, jaw slapping, open mouth displays, charging, and jaw clashing. Screams were produced during high-level aggression (n = 2 occurrences), typically when hippos were jaw clashing. Whines and groans were produced underwater when hippos were not visible. However, whines were mostly produced prior to or during low-level aggression (n = 15 occurrences). Groans were mostly produced prior to and following low-level aggression (n = 13 occurrences). Wheeze honks were produced by single individuals as well as by several individuals in temporal clusters (n = 4 occurrences), where they would surface and respond as a chorus. Wheeze honks choruses occurred in a variety of contexts in response to trucks driving by or during shifting of individuals in the mornings, whereas single wheeze honks were often produced during or following social interactions (n = 12 occurrences) (Table 11).

In the pulsed category, only growls were produced on the surface and were associated with dominance contexts and agonistic interactions. Growls were mostly produced in association with low-levels of aggression (n = 7 occurrences) such as open mouth displays, open mouth scoops, and jaw slaps and during interactions with another individual (n = 5 occurrences). Clicks were produced in association with food (n = 2 occurrences), and both clicks (n = 15 occurrences) and croaks (n = 1 occurrence) were produced underwater during a variety of social interactions when hippos could not be observed (Table 11).

Table 11. Descriptions and behavioral contexts of the acoustic signal types

Category	**Description**	**Medium**	**Sex (M, F)**	**Context**	**Concurrent behavior** (n = number of times acoustic signal occurred in context)
Burst of air					
Chuff	Short, repeated exhaled bursts of air	Air	M, F	Social-submissive	Retreat (n = 1) Follow low level aggression (n = 7)
Snort	Single, loud exhaled burst of air	Air/SAS	M, F	Social-dominance	Follow low and high level aggression (n = 4)
Bubble display	Exhaled burst of air underwater	Air/SAS	M, F	Social (?)	
Tonal					
Groan	Tonal signal with multiple harmonics, which decreases in frequency at the end	Underwater	M, F	Social-submissive (?)	Prior to low level aggression (n = 7) During interactions (n = 4) Following low level aggression (n =6) Follow high level aggression (n = 1)
Grunt	Tonal signal with few harmonics, short in duration, with an inverted u-shaped contour	SAS	M, F	Social-dominance	Prior to low level aggression (n =6) During low level aggression (n = 8) Follow low level aggression (n = 5) Follow high level aggression (n = 2)
Scream	Low frequency tonal signal, relatively flat contour, harsh	Air	F	Social-agonistic	High level aggression (n = 2)
Whine	High frequency tonal signal, may be one harmonic	Underwater	M, F	Social-submissive	Prior to approach (n = 4) During low level aggression (n = 11) Follow low level aggression (n = 4) Follow high level aggression (n = 1)
Wheeze honk	Wheeze followed by series of honks (2-9), often produced as a chorus of overlapping calls	SAS	M, F	Social	Temporal cluster (n = 4) During interactions (n = 2) Following low level aggression (n = 10)
Pulsed					
Clicks	Three or more pulses, either broadband or narroband	Underwater	M, F	Experiment, social (?)	Experiment (n = 1) Other (n =1) Social interactions (n = 15)
Croak	Series of low frequency pulses, duration less than	Underwater	M, F	Social (?)	During interaction (n = 1)
Growl	Series of low frequency pulses, duration more than	SAS	M, F	Social-dominance	Prior to low level aggression (n = 2) During interaction (n =5) During high level aggression (n = 2) Follow low level aggression (n = 5)

Discussion

The results of this study support previous findings that hippos produce a variety of acoustic signals in-air, underwater, and simultaneously in both mediums (Barklow, 1994, 1997, 2004). A majority of the signals were produced underwater (61%), which was similar to hippos in the wild (Barklow, 2004). Three main categories were identified representing burst of air, tonal and pulsed signals that could be produced separately in-air or underwater or as SAS signals. Both male and females produced sounds from these three categories, where tonal signals were the most frequent (67%) and females produced a majority of the signals (80%) (Figures 4.4 and 4.6). However, the females were housed as a social group of nine individuals, which could explain the higher occurrence of vocalizations due to the higher occurrence of social interactions. A few social interactions between the males were observed when they were housed in pairs or with 3 individuals. Each morning hippos were released from the barns to enter the pools where they spent their daytime hours. This shift to the pools simulated their natural behavior in the wild where they return to the water after foraging at night. As hippos enter the pools, they compete for space and most of their social interactions occur in the water (Eltringham, 1999; Karstad & Hudson, 1986; Klingel, 1991).

For the SAS signals, there seemed to be little difference in the acoustic structure of signal components produced in-air versus water. The main difference was that in the recordings, the underwater components preceded the aerial component, due to the rapid speed of sound transmission in water. As previously noted by Barklow (1997, 2004), underwater sounds also appear to emphasize higher frequencies, which may be due to the pathways for sound production. However, this may also depend or be due to the head position of the hippo and how much of the sound travels underwater. Previous studies have suggested that in-air sounds travel through nostrils, while underwater sounds may be transmitted through the mouth or the roll of fat around their throat (Barklow, 1997, 2004). However, the dissection of the female hippo mentioned in this study found no fatty tissue around her throat. Yet, it is known that hippos react to sounds on the surface and underwater, which suggests these sounds are not just an artifact of the loudness of the sounds or head position. Instead, SAS communication is likely to represent an important adaptation for hippos to respond to their surroundings above and below the surface.

In general, hippo acoustic signals were lower in frequency, which may be beneficial for propagation in shallow water. The optimum frequency for sound propagation is influenced by water depth, substrate type, and the sound-speed profile in marine environments where lower frequencies attenuate less (e.g., Jensen & Kuperman, 1983). Thus, hippos may have adapted to communication in the water by producing lower frequency sounds that attenuate less and travel farther distances, many of which border into the infrasonic range (below 20 Hz). None of the signals were solely infrasonic.

Analysis using the broad parameters of the acoustic signals such as the plot of peak frequency over bandwidth did not reveal specific information about the categories of sounds. These results suggested there were two broad categories: narrowband signals and broadband signals (Figure 4.5), but it is likely these measures were too broad to reveal additional signal types. Thus, I relied on visual inspection of spectrograms, aural analysis of sounds, and concurrent behavior to identify the different signal types and included additional measures on randomly selected signals within each category for further analysis. Comparing the mean frequency range of the signals in each category demonstrated that many of the signal types overlap and most of them are lower in frequency, below 3 kHz (Figure 4.17).

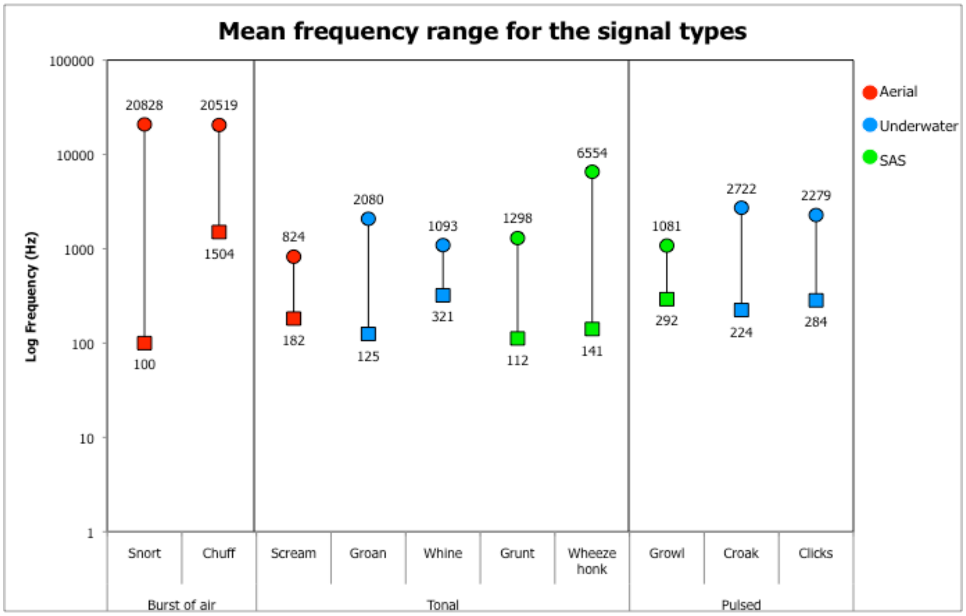


Figure 4.17. Mean frequency range for all signal types for male and female hippos.

The sections below provide summaries of the findings on the acoustic parameters of the different categories and sub-categories, as well as the behavioral contexts. The potential functions of signals will be discussed using basic principles of communication theory, which involve the signaler transmitting a signal that carries information that is received and interpreted by the receiver and results in a change in behavior (e.g., Marler, 1967; Rendall et al., 2009). To investigate the different signal types produced by hippos in close proximity, Morton's (1977) motivational and structural rules will be applied to discuss the contexts of calls. This concept assumes the structures of calls are conserved across species and can be used to indicate the motivation underlying the use of different calls (Morton, 1977). Harsh, low frequency sounds are associated with "hostile" contexts, while high frequency, tonal signals are associated with "friendly" contexts (e.g., appeasement, fright, or friendly approach) (Morton, 1977). Last, the potential mechanisms for sound production will be discussed based on the acoustic properties of the signals.

Bursts of air signals

For the burst of air category, three signal types were identified: chuffs, snorts and bubble displays (Table 3, Figure 4.7). Signals in this category were generally noisy bursts of air with no tonal components, suggesting they may be produced in the nasal system. Although snorts were primarily produced in-air, they could sometimes be detected underwater, which may be due to the loudness of the sound. Chuffs were only produced in-air and often included a bout of up to 8 chuffs. Snorts were lower in frequency and longer than chuffs, and were produced in association with dominance behaviors. The results of the PCA and leave-one-out cross validation tests provided further support for these signal types based on the differences in the frequency measures (minimum frequency, bandwidth, and peak frequency) (Table 4, Figure 4.8).

The low frequency and harsh sound of snorts was consistent with Morton's (1977) rule for hostile sounds. Chuffs were shorter and higher in frequency and associated with submissive behaviors that involved the hippo moving away from or in response to the display of a dominant individual (Tables 3 and 11). Chuffs represent a new sub-category that was only mentioned in a previous study as short exhaled bursts produced by hippos when prodded by dominant individual (Karstad & Hudson, 1986).

Although bubble displays were excluded from the acoustic analysis, the behavioral contexts warrant further discussion. Most cetaceans produce bubble bursts from the mouth or blowhole during a variety of different behavioral contexts that include foraging, play, excitement, protection, and aggression (e.g., Hills et al., 2011; McCowan et al., 2000; Reidenberg & Latiman, 2007b). In wild hippos, bubble displays were described as a display because they created a fountain effect on the surface and they were associated with social interactions (Barklow, 2004; Estes, 1991). Observations of these captive hippos suggested that bubble displays were associated with social interactions, but it was not clear whether they were related to dominance displays or excitement (Figure 4.18). These bubble displays contained infrasonic components and were often loud enough to be felt as vibrations on land. Similar to cetaceans, hippos may produce bubble displays to intimidate or threaten other individuals, or for protection or to avoid a dominant individual. Bubbles could also be used for buoyancy control, but this may not be necessary for hippos in shallow water.

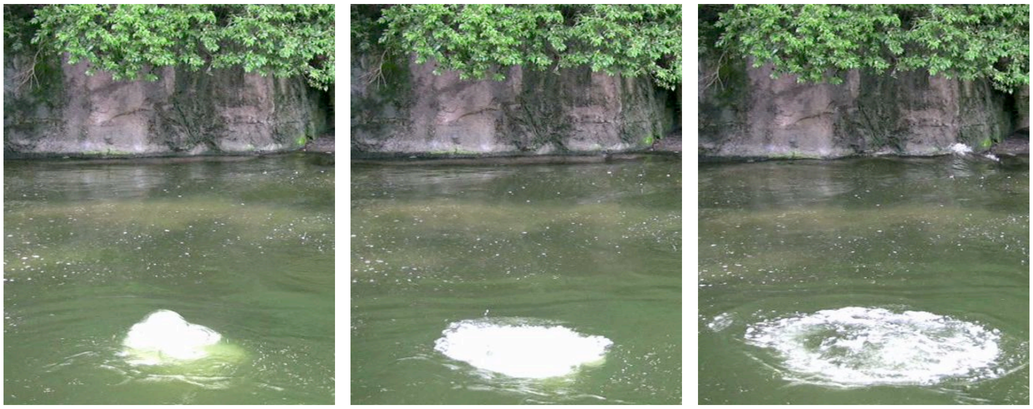


Figure 4.18. Stages of a bubble display produced by female hippos.

Tonal signals

The tonal category included 5 signal types (groans, grunts, screams, whines, and wheeze honks), which were mentioned in previous studies, but only whines were described in detail (Barklow 1997, 2004). These signals had a tonal quality (energy at a narrowband of frequency), with a clear fundamental frequency and some signals had multiple harmonics (Table 5, Figure 4.9), suggesting these sounds were produced in the larynx. Grunts, screams and wheeze honks were produced as SAS signals by hippos in the amphibious position. Whines and groans were produced underwater, which limited our ability to determine the exact behavioral context. On a few occasions, groans were also heard above the surface and screams were heard underwater, but this may have been due to the loudness of these sounds or the head position of the hippo.

The results of the PCA and leave-one-out cross validation tests provided support for three of the signal types (groans, grunts and whines) based on the frequency measures (minimum frequency, peak frequency, bandwidth, and fundamental frequency (F_0)) (Table 6, Figure 4.10). Groans differed from the rest of the tonal signals with a marked decrease in frequency at the end of the contour, which may suggest increasing hostility (Morton, 1977). Groans appeared to be associated with low-level aggression, potentially serving a dominance role, but the exact context could not be confirmed. Grunts were higher in frequency in the middle of the signal and lower in frequency at the beginning and end, forming an inverted u-shape or chevron. This chevron shape may indicate interest as described by Morton (1977), but observations of the captive hippos suggest they were associated with dominance behaviors (low and high-level aggression). Whines were higher in frequency than the other signal types and were associated with submissive contexts as previously reported by Barklow (1997) and consistent with Morton's descriptions of signals associated with appeasement (1977). Although whines were produced underwater when hippos could not be observed, they were associated with low-level aggression and were often produced prior to and during social interactions. This suggests whines may serve to appease a dominant individual. Screams may overlap in their acoustic parameters, but had a relatively flat contour with a harsh, metallic sound. Screams were observed during agonistic interactions with high-levels of aggression, such as when hippos were jaw clashing. However, it was not clear whether one or both of the hippos produced these signals during these interactions, but the structure of the sounds indicates hostility (Morton, 1977) (Figure 4.9, Table 11). The frequency parameters of the tonal signals need more fine tuned analysis and further observations of hippos are needed to confirm these behavioral contexts.

Wheeze honks were the most frequent call type produced by all individuals and as SAS signals, suggesting it functions as an important social signal. Although the basic structure of the call is similar above and below the surface, the transmission of this signal in both mediums appears to depend on head position of the hippo (Figure 4.11). When the hippo's head is above the surface, less sound travels underwater so the frequencies observed in the spectrogram may be reduced underwater. When in the amphibious position, sound appears to travel almost equally into the two mediums, but there are some differences. The peak frequency of the in-air components of wheezes was lower than the underwater components, and the in-air components of honks were lower for the minimum, maximum, peak, and fundamental frequencies (Tables 5 and 7). These are likely due to physical differences in the mediums in which these sounds are transmitted.

Wheeze honks were mostly produced as a chorus with overlapping calls from several individuals in a variety of contexts (Figure 4.11, Table 11). These observations provide support for the idea that wheeze honks function as a contact call produced by male and female hippos. Contact calls generally refer to signals that are used to determine the location and/or identity of other individuals in the social group. For example, elephants produce rumbles, which serve as contact calls to maintain social bonds across distances and can be used to determine the identity of the caller (e.g., McComb et al., 2003). Studies have described temporal patterns in the exchange of rumbles across short and long distances, which may include overlapping signals or antiphonal calling between affiliated individuals (e.g., Soltis et al., 2005). Rumbles were produced by elephants in several contexts such as when they were separated, reunited, or approached by a dominant individual (Soltis et al., 2005). A temporal pattern was observed in the production of wheeze honks by hippos that often started in the male barn, followed by males in the exhibit pools and then females in the exhibit pools (Table 11).

Similar to previous observations of wild hippos (Barklow, 1994; Estes, 1991; Feldhake, 2005; Walther, 1984), the dominant individual was the first to produce this call. Observations of these captive hippos suggest that the chorus may depend on who vocalizes first. If the dominant individual calls, other hippos are likely to surface and call in response. If a subordinate or young individual calls first, it is less likely that others will chorus. However, when multiple hippos were in the pool, it was not possible to determine the identity of the caller. Further research is needed to confirm these findings, but these observations provide support for the significance of the wheeze honk as a contact call important for social cohesion and communication within and across groups.

Pulsed signals

Three signal types were included in the pulsed category: clicks, croaks, and growls (Table 9, Figure 4.12). This study did not include inharmonic clicks or tooth clashes reported by Barklow (1997) since the pulsed signals were classified as having three or more pulses. It is unclear how hippos produce discrete pulses, but it possible they may use mechanisms in the nasal system similar to cetaceans. Growls were produced as SASs, while clicks and croaks were produced only underwater. The results of the PCA and leave-one-out cross validation tests provided support for these signal types based mainly on differences in the interclick interval (ICI), duration of the pulses and duration of the signal, but also on the frequency measures, although there was some overlap (Table 10, Figure 4.13).

Croaks were shorter in duration, while the duration of clicks and ICI of click trains were longer than either croaks or growls. Broadband and narrowband click trains were produced during the pilot study and experimental sessions with male hippos, suggesting they may be used for echo-ranging (*See Chapter 5*). Female hippos produced clicks and croaks during the sessions with carrots and baseline sessions, suggesting they may be associated with social interactions or echo-ranging (*See Chapter 5*). Adult hippos produced croaks in different contexts (Table 11), which contrasted previous reports by Barklow (1997) where he suggested they were produced when calves and subadults were interacting. However, as Barklow (1997) suggested, they may be associated with alertness or excitement. Growls were associated with dominance behaviors such as approaching or charging another individual (Table 11). Growls were lower in frequency and harsher in sound, indicating hostile contexts (Morton, 1977). Croaks and growls may be similar to the burst pulse sounds produced by dolphins (e.g., Au & Hastings, 2008), which are generally associated with excitement and agonistic interactions.

Although the analysis of the acoustic parameters supports the subcategories, there were many occurrences of graded signals and overlapping signals that were excluded from this analysis (except for wheeze honk choruses). For example, click trains often graded into a whine or were interrupted by chewing or other noises. Several signals such as groans and clicks were often produced with bubbles or bubble displays, but it remains uncertain as to whether this is due to emission through the nostrils or part of a display. These graded signals could represent variations of the above-mentioned calls or they could suggest multi-element calls that were important for social communication, but further research is needed.

Sex differences

Male and female hippos produced acoustic signals from all of the categories (burst of air, tonal and pulsed), yet many of the signal types were rarely produced by males (e.g., whines, groans, croaks) and screams were not recorded from the males. The increased number and diversity of acoustic signals produced by females (Figures 4.4 and 4.6) may be due to the larger group size or differences between sexes. Wheeze honks were frequently produced by both sexes, so these signals were investigated to determine possible sex differences in the acoustic parameters. Although there were some differences between the in-air and underwater components, there may be other differences between the sexes. The comparison of the in-air and underwater components demonstrated that the wheezes and honks produced by females were lower in frequency than the males (Figure 4.11, Table 8). Although the calls of the males were predicted to be lower in frequency, this difference may be due to age differences rather than sex. Two of the males were younger in age (4-6) as compared to the majority of the females (9-35).

Thus, further comparisons are needed to determine whether sex or age differences may occur in the types of vocalizations.

Captive versus wild hippos

The recordings from these captive hippos appear to be similar to those recorded from other researchers in the wild (Barklow, 1994, 1997, 2004; Krauss, personal communication; Solomon, personal communication) and in captivity (Kreuger, 2000). The tonal whines described by (Barklow, 1997) ranged from 600 Hz to 7000 Hz and were approximately 1 to 6 seconds long. The whines in this study ranged from 321 to 1093 Hz, lasting around 0.7 seconds. Although these were lower in frequency, it is likely that the category described by Barklow (1997) may have encompassed groans and the other tonal signal types described in this study. Barklow (1997) reported that croaks ranged from 15 Hz to 50 Hz and were approximately 0.19 seconds long. The croaks in this study were similar in duration (mean = 0.245 s), but were higher in frequency, ranging from 224 to 2722 Hz. Barklow (1997) reported that narrowband clicks were below 2 kHz and wideband click ranged from 250 to 10 kHz, lasting from 0.5 to 2 seconds long. In this study, click trains (narrowband and broadband) generally ranged from 284 to 2279 Hz and were 0.54 seconds long (*See Chapter 5 for more details*).

Limitations

Although these findings were similar to previous studies, there were some factors that need to be considered in the interpretation of these results. Most of the recordings were made of the hippos in the early morning hours, prior to the facility opening to the public when the pool filters could be turned off. A few sessions were conducted in the evening when the hippos were returning the barns, but these recordings were only made on land. The hippos were also separated into groups by sex where the nine females were housed together, but the males were either alone or in pairs in the pools.

In addition, I could not determine which individuals were producing the acoustic signals. Each group was observed as a whole and although I tried to pan the camera to record interactions, the hippos were often out of sight. It was also not possible to determine the exact type of behavioral interactions when hippos were submerged in the murky water. The location of the hydrophone was fixed so it could be monitored during the recordings, which limited the viewing and recording range. Thus, amplitude measures were excluded from this analysis because the distance and orientation of the hippos to the hydrophone could not be determined.

Further research

Future studies of the acoustic communication of hippos should include recording sessions throughout the day and night to determine whether there are changes in call rate and types of vocalizations. Although chuffs are produced only in-air, no acoustic signals have been recorded that were only produced on land. Additional cameras and microphones could be used to record from different areas of the pool to be able to localize the hippos. Further research is needed on the acoustic parameters of the acoustic signals to provide more details about the frequency modulation of the signals over time.

For example, further investigations of the tonal signals could include examinations of the acoustic structures of hippo signals in comparison to those produced by cetaceans to explore the evolution of these signals. Research by May-Collado and colleagues (2007) has demonstrated that sociality influences the evolution of complex tonal signals in cetaceans. They reported that increasing complexity in the features of tonal signals (e.g., minimum frequency and duration) was related to changes in group size and the evolution of social complexity. Thus, the diversity of tonal signals produced by hippos and their association with social interactions suggest similar patterns to cetaceans in the evolution of these signals.

In terms of the behavioral contexts, mixed social groups would also be beneficial to record to investigate the variety of social interactions between individuals of different ages and sex. Additional recordings could also determine possible sequences of sounds that were not analyzed in this study. Recordings of hippos in clearer water would help to provide information about interactions when hippos are submerged. Playback studies could be also used to determine the functions of signals based on the responses of the receivers. Focal samples of individuals could be used to identify which hippos were producing the acoustic signals to provide more insights about possible differences between the sex, age, and status of the individuals. Other communication theories such as honesty in signaling can also be used to examine the functions of hippo acoustic signals. The question of honesty in communication concerns the reliability of the information conveyed in a signal (e.g., Searcy & Nowicki, 2005). Further research could apply this principle to investigate the reliability of information conveyed in dominance and submissive contexts based on which individual is calling and to examine the responses of individuals to the calls.

Conclusion

The results of this study have built on previous research to further describe the acoustic and behavioral repertoires of the hippopotamus. Three main categories were described which included burst of air, tonal and pulsed signals. Within these categories, eleven distinct signal types were described and differentiated by their acoustic parameters and behavioral contexts. These findings provided more information about the functions of different signals and their potential significance to the social group, particularly in mediating interactions between dominant and subordinate individuals. Further research is needed to continue to describe the signal types, functions and mechanisms of sound production, including how these signals compare to those produced by their close aquatic relatives, the cetaceans.

Chapter 5: Evidence of echo-ranging by the hippopotamus

The recent molecular, genetic and fossil evidence for a hippo-whale clade (Geisler & Theodor, 2009; Spaulding et al., 2009) raises questions about the adaptation of the sensory systems of the common hippopotamus (*Hippopotamus amphibius*) to an amphibious existence. Do hippos possess a rudimentary form of echo-ranging that provides enhanced detection and navigation in the murky waters of the African rivers and lakes they inhabit? Hippos give birth and nurse in the water and most of their social interactions occur in the water (Eltringham 1999; Feldhake, 2005), but they are less specialized (e.g., body shape, adaptations for swimming and diving, etc.) than marine mammals for aquatic living. However, relatively little is known about the sensory systems of hippos and more research is needed to elucidate the degree to which they have adapted to amphibious conditions.

The idea that the amphibious hippo might use echolocation was first suggested by Longhurst (1966) after he observed hippos in the wild. He suggested that they might use echolocation in murky water as they appear to scan their environment underwater. Although hippos are typically in the water during the day, they are also occasionally in the water at night. In addition, there are reports of hippos foraging on aquatic vegetation (Mugangu & Hunter, 1992) and other animals such as impalas (Dudley, 1998), which suggest echo-ranging may also be advantageous for searching for food or other objects in the dark or murky water.

A few studies have reported the production of click trains by wild hippos (Barklow 1994, 1997, 2004; Krauss, personal communication) and captive hippos (Kreuger 2000; Barklow 1997). Hippo click trains are produced only underwater and resemble echolocation signals produced by their close aquatic relatives, the cetaceans. Barklow (1997) described these signals as wideband, narrowband, or inharmonic clicks (single pulses without harmonic components) that were speculated to be used for social communication. One experimental study tested the possible function of hippo clicks using a navigation and obstacle avoidance task similar to earlier studies with dolphins and bats (Krueger, 2000). Three captive hippos (1 male, 2 females) were tested when alone in the pool on moonless nights and when aluminum sulfate was added to cloud the water. Hippos were tested under three conditions: control condition (normal pool, pre and post conditions) versus experimental conditions (with 7 plastic barrels placed in the pools) to determine whether they would navigate around the barrels and whether there was an increase in click production. Many clicks were recorded, but there was no difference in the number of click trains produced across the different conditions. Thus, the researchers did not conclude that clicks were used as a form of echolocation, but the production of clicks by solitary hippos was provocative (Krueger, 2000).

Hippo click trains may represent a more rudimentary echo-ranging system than found in dolphins because they are amphibious and not as specialized for aquatic living; yet click trains may be used for orientation and detection of objects, conspecifics, their calves, and potential predators in the murky water they inhabit. Discovering the use of a rudimentary form of echo-ranging in hippos would be a significant finding and could provide evidence for the possible convergence of this sensory system in cetaceans and hippos.

Echolocation

Echolocation is the biological sonar system used by animals, which involves the production of acoustic signals that are transmitted into the surrounding medium. The sound waves reflect off of objects and produce an echo that is received and processed by individuals to obtain an “acoustic picture” their environment (Au & Hastings, 2008). Although researchers such as Spallanzani and Jurine began questioning how bats could “see” in the dark in the late eighteenth century, it was not until 1938 that Pierce and Griffin discovered their use of ultrasonic signals while flying (Au, 1993). Later research by Griffin and Galambos confirmed that bats could hear ultrasonic signals, leading Griffin in 1944 to apply the term “echolocation” to describe orientation and detection systems in animals (Au, 1993).

Research in bats paved the way for hypotheses about echolocation in cetaceans. Mcbride (1956 cited in Au, 1993) first suggested their use of echolocation in the late 1940s, followed by other researchers such as Wood (1953), Schevill & Lawrence (1956), and Kellogg (1958 cited in Au, 1993) who provided experimental evidence that dolphins could detect objects underwater and were producing wideband pulses. However, research by Norris et al. (1961) provided the first conclusive evidence of the use of echolocation by dolphins using eyecups to demonstrate that a blindfolded dolphin could still navigate, detect, and discriminate objects in a pool.

Since these early studies, many researchers have demonstrated that odontocetes (toothed whales and dolphins) and microchiropteran bats have evolved highly specialized echolocation systems. These species use biosonar for navigation, orientation, foraging, object detection, and object discrimination in various environments where visibility is limited (Au & Hastings, 2008; Grinnell & Griffin, 1958; Houser et al., 1999; Norris et al., 1961). Experiments to test these abilities have involved a variety of masking studies and object detection or avoidance tasks to determine the capacity of the bats and dolphins to detect stationary and moving objects of different sizes and to use echolocation for navigation and orientation (Au, 1993; Au & Hastings, 2008; Grinnell & Griffin, 1958; Norris et al., 1961).

In the case of dolphins, echolocation clicks, or click trains, are generally described as sequences of broadband pulses (ranging from 20 Hz up to 200 Hz), short in duration (40-70 microseconds), with peak frequencies between 120 and 130 kHz (Au, 2004; Au & Hastings, 2008; Norris et al., 1961). Clicks tend to be produced as high amplitude, wide-band signals that include a broader frequency range, which can provide more information from the environment. The shorter wavelengths of high frequencies reflect better from small objects and provide more resolution for object discrimination, while the longer wavelengths of lower frequencies travel farther, have less attenuation and reflect better from larger objects for navigation (Au & Hastings, 2008). Dolphins can control the timing of clicks produced as clicks trains, which are sequences of click pulses, by varying the click intervals or duration between each click (Au & Hastings, 2008). The delay in time and changes in intensity of the returning echo enable dolphins to determine the distance and direction of objects (Jones, 2005). As dolphins hone in on a target, the intervals between clicks decrease, creating a buzz like sound (Au & Hastings, 2008). Dolphins may also be able to modulate the peak amplitude of clicks depending on the noise level of the environment, the individual dolphin and the type of task (Houser et al., 1999).

Several other marine mammal species have been investigated to determine whether they have evolved similar sonar based systems as a result of the analogous environmental pressures and adaptations to aquatic living. All odontocetes and species such as pinnipeds and baleen whales (mysticetes) produce burst pulse signals, which may not function as echolocation clicks. These signals are distinguished based on the frequencies, interclick intervals and the behavioral contexts in which they are used (Au & Hastings, 2008). Pulsed signals such as the codas of sperm whales and clicks produced by other baleen whales tend to be lower in frequency than echolocation clicks and have instead been hypothesized to function as social signals (Au & Hastings, 2008). Perhaps due to the similarity between echolocation clicks and other burst pulse signals, less research has been conducted on the parameters and possible function of the latter (Au & Hastings, 2008; Schusterman et al., 2000).

However, one recent study by Stimpert and colleagues (2007) provided evidence that humpback whales might use a form of biosonar. They discovered that these baleen whales emitted "megapclicks" concurrent with feeding behaviors at night. They reported that the dominant energies of these clicks were below 2 kHz, but the click interval properties were similar to dolphins. Although these clicks were lower in frequency, the longer wavelengths could reflect off larger objects and may still provide information about environment. Other baleen whales such as blue and minke whales produce pulsed sounds that may be used in social communication, but it is unclear whether or not these signals may also be used as a form of echolocation (Au & Hastings, 2008).

In the case of pinnipeds, there is some debate about whether or not they use a form of echolocation, but in general, it has been argued that the pulsed signals they produce do not represent an active form of biosonar (e.g., Schusterman et al., 2000). Pinnipeds have evolved a semi-aquatic lifestyle and are adapted to communicate in both water and on land, though there may be some differences based on the lifestyles of the different pinniped species. They forage in the water, but give birth and nurse on land, which may partially explain why they are not as specialized for the marine environment. Several experiments have been conducted that did not support the echolocation hypothesis and the investigators have instead suggested that pinnipeds evolved other adaptations such as better underwater hearing and vision to detect objects in their environment (Au & Hastings, 2008; Schusterman et al., 2000).

Odontocetes and microchiropteran bats are considered to have sophisticated echolocation systems, but there are differences in how these signals are produced and detected based on the environments they inhabit. While flying at night, bats produce echolocation calls that are transmitted in air where sound travels about 5 times slower (340 m/s) than it does in water (1500 m/s). As a consequence, bats use echolocation signals for detection of objects at a closer range, primarily for orientation and for the detection of insects (Au, 2004). Their echolocation calls have been classified as either frequency-modulated (FM) or constant-frequency (CF). The FM signals may be produced alone or with CF components and are typically short in duration ranging from 0.3 to 300 ms (Au, 2004). Most bats produce echolocation signals via their larynx (Brown & Grinell, 1980), while dolphins produce clicks via their nasal system (Cranford et al., 1996). Like odontocetes, bats adjust their emission rate in order to receive echoes before the next pulse or call is produced (Au, 2004)

In addition to odontocetes and bats, a few other species including night-active birds (oil birds and cave swiftlets), rats, shrews, and tenrecs have been reported to produce pulsed or frequency modulated, broadband signals suggested to be used for echolocation (Buchler & Mitz, 1980; Thomas & Jalili, 2004). Although these species may not use echolocation to find fast-moving prey, they live in conditions with limited visibility where a form of echolocation may be useful for orientation and object detection. For example, a recent study demonstrated that shrews produce high-pitched laryngeal twitters (broadband, frequency modulated and multi-harmonic calls) when in new environments. Call rate was influenced by substrate density, rather than the presence of conspecifics, suggesting an echo-based system that may be useful for close spatial orientation (Siemers et al., 2009). While there still appears to be some controversy over whether these species have evolved specialized echolocation systems, it is possible these signals represent forms of echolocation that are advantageous for basic orientation, navigation and object detection at short distances.

Research goals

The purpose of this study was to investigate whether hippos use clicks as echo-ranging signals. Using observational and experimental methods, solitary and paired male hippos were tested on an object detection task to eliminate the possibility that clicks serve a social function and to determine whether they would produce click trains underwater while searching for food items, whether hippos would produce more clicks in murky or dark conditions, and whether there was any difference in the click trains they produced.

Methods

Subjects and recording equipment

The subjects of this study were five male hippopotamuses (*Hippopotamus amphibius)* ranging from 5-12 years old (at the start of the study) at Disney's Animal Kingdom® (DAK) in Lake Buena Vista, Florida. Recordings were made of male hippos on exhibit in the river pool (approximately 230 ft long with variations in width up to 75 ft wide, depth ranging from 0.5 to 2.7 m) and underwater viewing pool (65 ft long and 65 ft wide, depth ranging from 0.3 to 3.2 m) (Figure 5.1). Simultaneous video and audio recordings were collected during summer months (pilot study-one week in August 2007, June-August 2008 and July-August 2009), using a Vixia HV20 Canon digital video camera, C54XRS Cetacean Research Technology Hydrophone (.008-310kHz (+1/-20dB)), QTC 50 Earthworks microphone (.003-50kHz (+1.5dB)) and HD-P2 Tascam digital audio recorder (.02-20kHz (-1.0dB), 44.1 kHz sample rate).

Figure 5.1. Male hippo exhibit pools: A) river pool, B) underwater viewing pool.

Pilot study (2007)

Prior to developing the methods for the experimental sessions, a pilot study was conducted in August 2007 to test whether certain food items would sink and to determine whether hippos would search for food items underwater. The hippos occasionally received food items such as lettuce and banana leaves as enrichment in their pools and on the shore. Hippos were previously observed eating food in the pools, sometimes sinking as they continued eating. During the pilot study, three male hippos were recorded (one session, one hour in duration) in the exhibit pool at night when carrots, cantaloupe, and a watermelon were offered; only the carrots sank. Although I could not confirm that the hippos ate the carrots, they did appear interested in them. No other night sessions were conducted due to scheduling issues.

Baseline sessions (2007 and 2008)

Opportunistic audio and video recordings were collected from male hippos in the exhibit pools six days a week (Monday-Saturday) between the hours of 5:30 and 8:30 A.M., prior to the facility opening to the public and when the filters were turned off. Ten baseline sessions, approximately one hour in duration were conducted to record the ambient noise, acoustic signals, and concurrent behavior of solitary (n = 2 sessions) and paired (n = 8 sessions (one session was with 3 males)) male hippos in the exhibit pools. Prior to the beginning of each experimental session, a few minutes were recorded prior to the hippo coming out and when the hippo was in the pool before carrots were offered.

Conditioning procedure (2008 and 2009)

For the experimental trials, hippos were trained on an object detection task to determine whether they would produce click trains while searching for carrots underwater. The original plan was to test two males and two females that were food motivated. The female hippos were not as food motivated as the males and could not be isolated, so only the males were tested. Four of the five male hippos were first conditioned in their inside holding areas to associate a novel, 1-2 kHz tone played in-air using a stereo with the presentation and delivery of carrots and to elicit search behavior. The hippo team (zookeepers working with the hippos) conducted these initial training sessions three days a week, one month prior to the start of the summer recording seasons (May 2008 and June 2009). Team members played the tone (once per presentation of carrots), first presenting the carrots directly to each hippo and then progressively moving the carrots further distances from the hippos in their holding areas. At the beginning of the recording season (June 2008 and July 2009), their behavior was observed in the barns during training and once the hippos exhibited anticipatory search behavior in response to the tone, the procedures were conducted with isolated hippos in two outdoor exhibit pools. The hippos were isolated to rule out the potential social context of clicks.

Several pre-experimental sessions (n = 10) were conducted with each of the male hippos to determine whether they would eat carrot bundles on land and in shallow water. Hippos were first tested on land when they were going out on exhibit, and then carrot bundles were progressively moved to the edge of the water, shallow water, and ultimately deeper water. Once it was confirmed that the hippos would search for the carrot bundles in deeper water, hippos were tested in the area on the other side of the islands where the water was deeper (approximately 3 m). Three of the males did not search for carrots, so a fifth hippo (Biko) was included later, but was not pre-conditioned and only tested in the underwater viewing pool (exhibit pool).

Experimental sessions (2008 and 2009)

Experimental sessions (n = 68) were conducted in a similar manner as the baseline methods. Several sessions began before dawn and with the exhibit lights turned off in attempt to minimize visual cues. At the beginning of each session, one male hippo was released from the barn in the early morning and typically went right into the pool. The audio and video recordings were started before the hippo was in the pool to record each male in the "pre-search condition," which was defined as when the hippo was in the pool before carrots were offered. An experimental trial began either 1) when the hippo came over to the middle of the pool or 2) the tone was played and carrot bundles were tossed into the pool to attract the hippo's attention.

Sound transmission was tested in different parts of the pool and it was determined that the configuration of the concrete islands prevented transmission from one side of the river pool (Figure 5.1). However, the resonant frequencies were not tested due to the complex design of the pools and difficulties determining the distance of the hippos to the hydrophone. To ensure sounds would be recorded by the hydrophone, carrot bundles were tossed into the middle of the river pool near where the hydrophone was placed.

For the experimental sessions, bundles of 3-5 large carrots that were tied together using either banana leaves or corn husks were offered each experimental trial. Carrot bundles were tossed into the pools at random locations within the middle area of the pool to determine whether the hippos would search for the carrots and whether they were producing click trains while searching underwater. Each experimental session included approximately four presentations of carrot bundles (depending on the availability of carrots).

Two males, Hans (6 year old) and Henry (12 years old), were tested in 2008 and three additional males, Padron (7 years old), Uzazi (7 years old), and Biko (5 years old), along with Hans from the previous summer, were tested in 2009 (Total number of sessions per hippo: Hans = 45, Biko = 13, Henry = 0 (stopped responding after pre-experimental sessions), Padron = 6, Uzazi = 4). Only two males, Hans and Biko, were responsive to the object detection task and were motivated to search for carrots. Success in finding carrots was determined when either chewing was heard underwater or when the hippos came to the surface to consume them and carrots were visible in their mouths. During the second season (2009), the protocol was modified so that the number of trials per session was increased from 3 to 5 and a few sessions (n = 6) were conducted when carrots were placed in the pool prior to releasing Hans from the barn to remove possible visual and acoustic cues from splashing.

During the experimental sessions, solitary hippos were tested to eliminate the possibility that click train production was due to social factors. However, on a few occasions, Hans would not come out of the barn so Henry was also released into the pool. Henry and Hans were normally housed together in the river pool during the day and Henry was often released towards the end of the experimental sessions, which sometimes meant he was in the pool while Hans was still searching for carrots. Henry was in the pool at some point during 22 of the 45 experimental sessions with Hans, yet Henry almost always stayed on the other side of the islands. However, Henry's presence in the pool could have had an influence on these sessions.

Pseudo-experimental sessions (n = 8 sessions with 9 female hippos) were also conducted with the group of female hippos in 2009. During these sessions, the females were offered individual or bundles of carrots (without prior training) to determine whether they would search for the carrots and whether they produced clicks and/or other pulsed signals while searching. The goal of these sessions was to be able to compare the occurrence and parameters of the click trains produced by females to those produced by the males during the experimental sessions. Prior to the carrots being tossed into the pool, a few minutes were recorded to let the hippos settle as they entered the pool, unless they were already on exhibit.

In addition, the levels of turbidity in the male river pool were measured to determine whether increased turbidity related to an increase in the occurrence of click trains. The underwater viewing pool was always clear to the bottom, while the river pool was murky and larger in size. However, water clarity improved during the second season. A Secchi disk (Hou et al., 2007) was used to measure levels of turbidity to provide an estimate of visibility underwater. Turbidity was not measured every day, but visibility was sampled a few times during the experimental sessions to determine changes in turbidity related to the increase in sunlight after dawn.

Data Analysis

The acoustic parameters of the click trains were measured using Raven acoustical analysis software (version 1.3). Click trains were defined as a sequence of three or more pulses that were short in duration (less than 0.03 seconds). Spectrograms were generated and examined visually to measure the minimum and maximum frequencies, click duration (duration of each individual pulse), duration of click train (onset of first click to end of last click, <0.3 seconds from other clicks), peak frequency (frequency with highest amplitude), number of clicks, and interclick intervals (ICI, duration between clicks) of each signal (Spectrogram parameters: Hanning window size = 1586 samples, 3db filter bandwidth = 40 Hz, overlap = 90%, frequency grid DFT size = 2048 samples)). Amplitude was not included because I could not measure the distance or orientation to hydrophone when the hippo was moving underwater. Experimental sessions were analyzed to determine whether click trains were produced prior to carrots being offered versus while hippos were searching for the carrots. Search behavior was identified as target-oriented movement underwater that occurred after carrots were offered and the recorded underwater or surface chewing sounds were used as an endpoint. To compare the mean acoustic parameters, click trains (males n = 35, females n = 35) with good signal to noise ratios were selected and separated based on mean peak frequency. The acoustic parameters were compared using independent t-tests in PASW (version 18.0). A logistic regression test in PASW (version 18.0) was used to examine whether turbidity, time of day (pre or post dawn), presence of another hippo, and whether carrot bundles were placed in the pool prior to the start of the session may have influenced the occurrence of click trains.

Results

Pilot study (2007)

In the pilot study, eight click trains were produced when three hippos were underwater during the night session (one hour in duration) when food items were offered. Although I could not confirm that the hippos were searching or obtained the carrots, no clicks were recorded prior to the carrots being tossed into the pool. Due to the fact that three males were in the pool, the possible social function of clicks trains could not be ruled out because there were many social interactions between the three males. In addition, the filter could not be turned off during this session, so the click trains could not be accurately measured and were excluded from the statistical analysis.

Baseline sessions (2007 and 2008)

No click trains were recorded from solitary or paired male hippos during baseline recordings (n = 10 baseline sessions of hippos under normal conditions) in 2007 or 2008.

Experimental sessions (2008 and 2009)

A total of 68 experimental sessions were conducted with the five males when carrots were offered. One of the four pre-conditioned hippos (Hans) and the one unconditioned hippo (Biko) were highly motivated to search for carrots and produced click trains in this context. The other three hippos displayed neither search behavior nor clicking.

Hans and Biko produced 51 click trains during 28 of their 58 experimental sessions. Hans produced 27 click trains when alone (across 17 sessions) and 22 click trains when paired with another hippo (across 9 sessions). Biko produced 2 click trains when alone (across 2 sessions). The majority of click trains (57%) were produced when hippos were alone.

Both hippos seemed to become more responsive to the object detection task as the weeks progressed as measured by increases in their search behavior and success in getting carrots. The number of click trains that occurred over successive experimental sessions was plotted for each summer, revealing a slight increasing trend over time (Figure 5.2). Both Hans and Biko searched for and obtained carrots underwater and then rose to the surface and finished eating. Biko remained underwater longer while chewing than Hans.

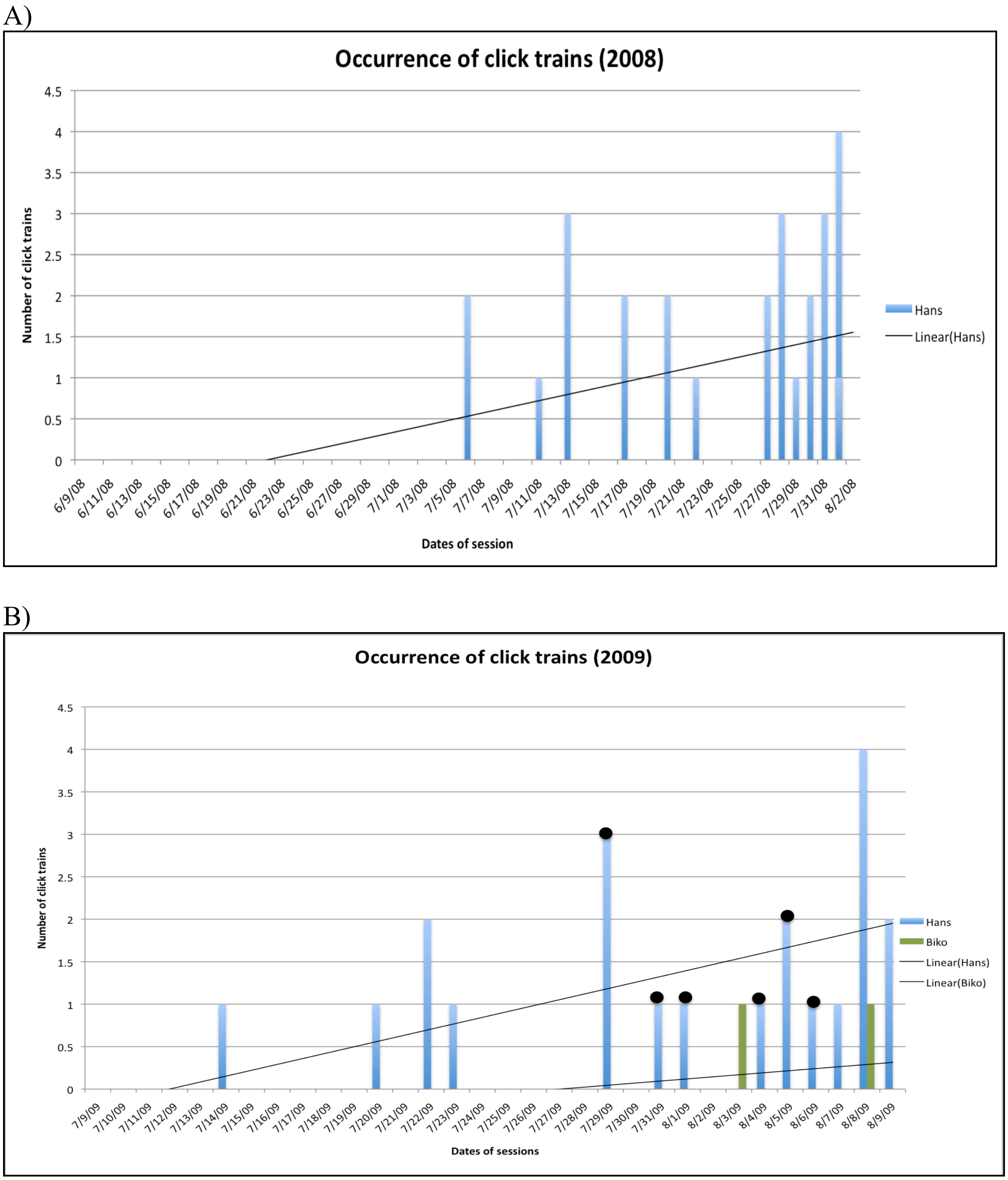


Figure 5.2. Frequency of occurrence of clicks trains produced during search contexts across successive experimental sessions in A) 2008 and B) 2009. Sessions with carrots pre-placed in the pool are denoted with the black circle. All sessions are shown.

No click trains were recorded outside the search context (e.g., when hippos were not searching) from any of the five hippos. Most click trains occurred less than a minute (n = 28 click trains) and between a one to five-minute interval (n = 14 click trains) prior to obtaining carrots (9 click trains were produced after a five-minute interval), demonstrating that clicks coincided with searching behavior (Figure 5.3).

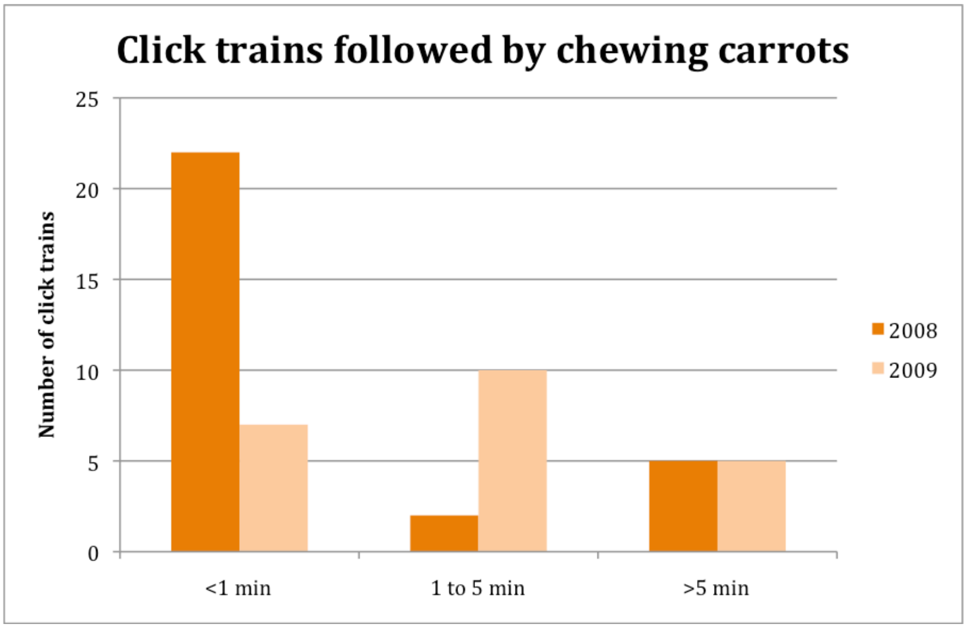


Figure 5.3. Click trains occurring less than a minute, between 1 and 5 minutes, and over a five-minute interval prior to chewing carrots underwater or on the surface.

Although click trains were not produced in every session, autocorrelations were run to ensure independence of the data for comparisons of the mean acoustic measures. The acoustic parameters were measured for a subset of the male hippo data (35 of the 51 signals) with the best signal-to-noise ratio. Visual inspection of the spectrograms initially suggested two types of click trains based on bandwidth, narrow band and wide band clicks (Figure 5.4). To determine whether there were different types of click trains, the acoustic parameters were plotted for each click train and peak frequency demonstrated two clusters (Figure 5.5). Using this parameter, the click trains were grouped into two categories: broadband click trains (n = 10, peak frequency above 1000 Hz) and narrowband click trains (n = 41, peak frequency below 1000 Hz). The results of the independent t-tests demonstrated significant differences between the two types of clicks trains based on the maximum frequencies ($t(33) = -5.231$, $p < 0.001$) and peak frequencies (t(33) = -9.550, $p < 0.001$) (Table 12). The click durations and ICI did not vary across the signals, but the broadband click trains contained a wider bandwidth, higher peak frequency, were longer in duration and had more individual clicks than the narrowband click trains; yet the broadband clicks trains also included individual clicks that varied in bandwidth (Figure 5.4).

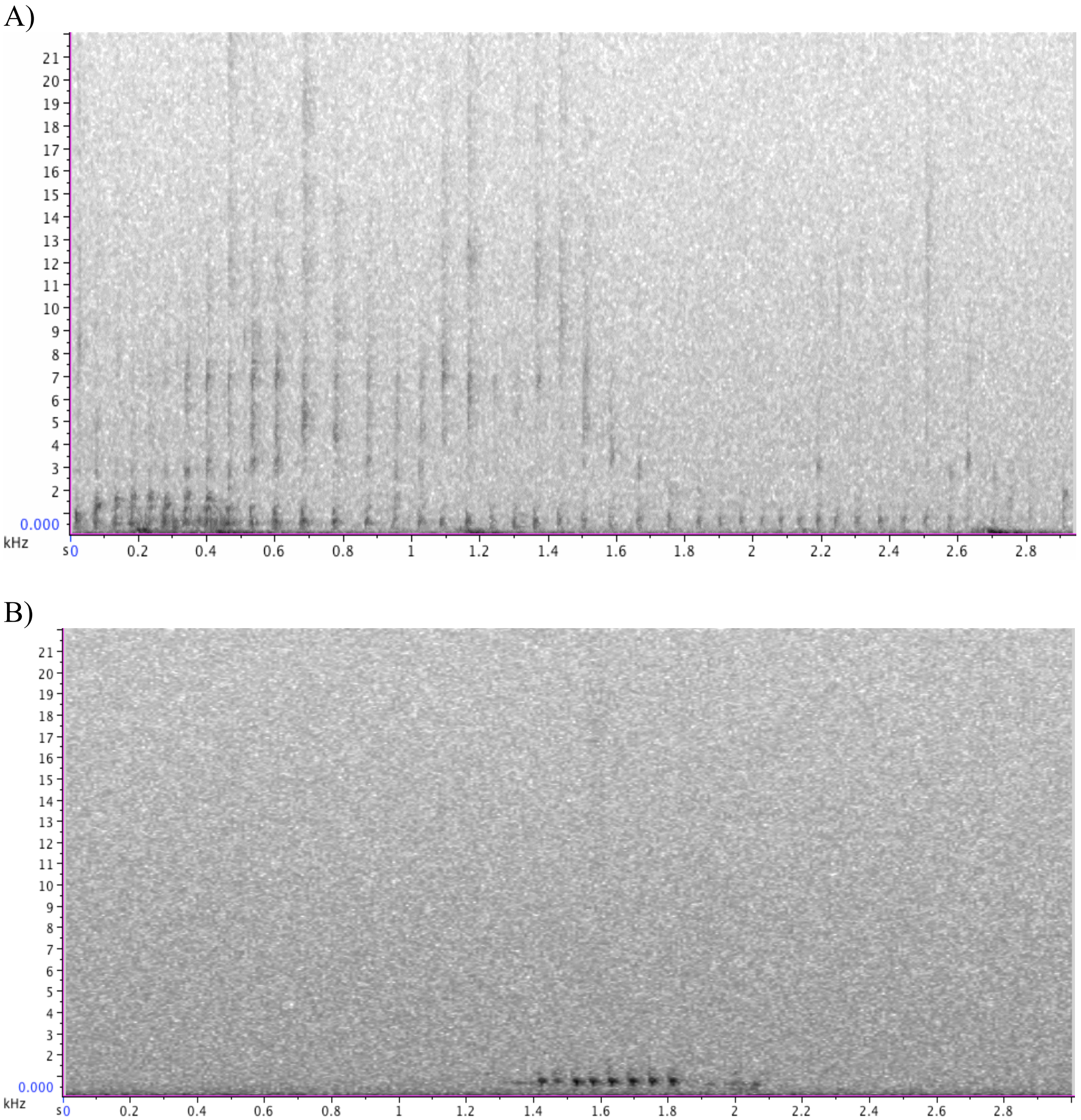


Figure 5.4. Spectrograms of A) a broadband click train and B) narrowband click train from Hans while searching for carrots underwater.

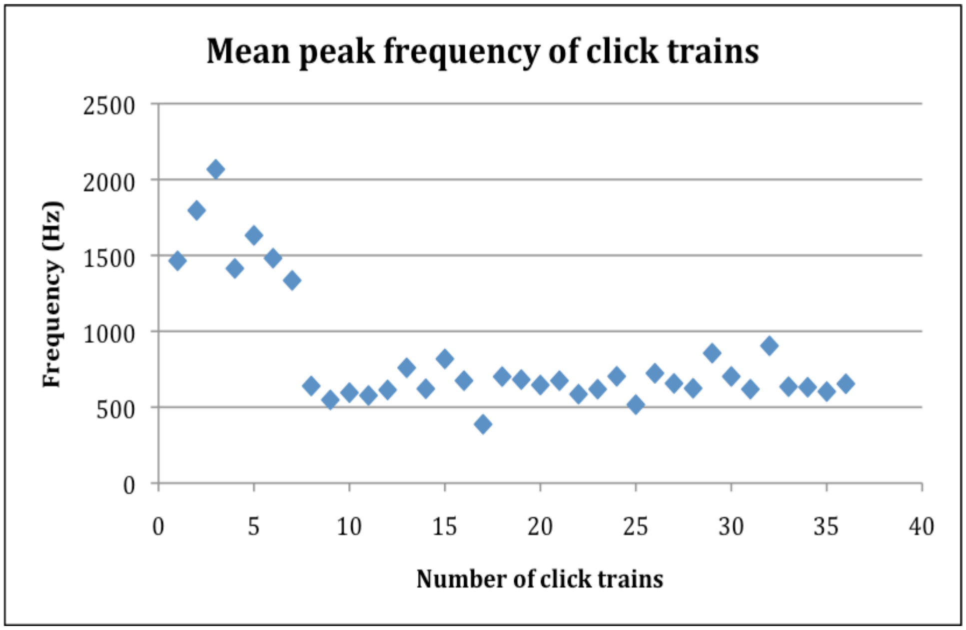


Figure 5.5. Plot of mean peak frequency for click trains produced by male hippos.

Table 12. Acoustic structures of male click trains (n = 35) (*p < 0.05)

	Minimum frequency (Hz)	**Maximum frequency (Hz)**	**Click duration (s)**	**Peak frequency (Hz)**	**Duration (s)**	**ICI (s)**
Narrowband clicks	352±84	2683±3106*	0.017±0.005	654±102*	0.659±0.702	0.059±0.002
Broadband clicks	474±162	9802±3692*	0.016±0.004	1599±257*	1.10±1.25	0.060±0.010

The occurrence of click trains during the experimental sessions with males and pseudo-experimental sessions with females demonstrated an increase in the occurrence of click trains when carrots were offered. Two males produced 51 clicks trains during 58 experimental sessions (mean = 0.88 click trains), and no click trains were recorded during baseline sessions or outside the experimental trials. Females produced 88 click trains during eight sessions (mean = 11 click trains) when carrots were offered. Although females produced 96 clicks trains during the 51 baseline sessions when carrots were not present (mean = 1.88 click trains), the frequency of occurrence of click trains was lower than when carrots were present (Figure 5.6). Results of a Fisher's exact test demonstrated significance differences in the production of click trains across baseline and experimental sessions for the males and the females ($p < 0.01$), except for the comparison of males and females during the experimental sessions.

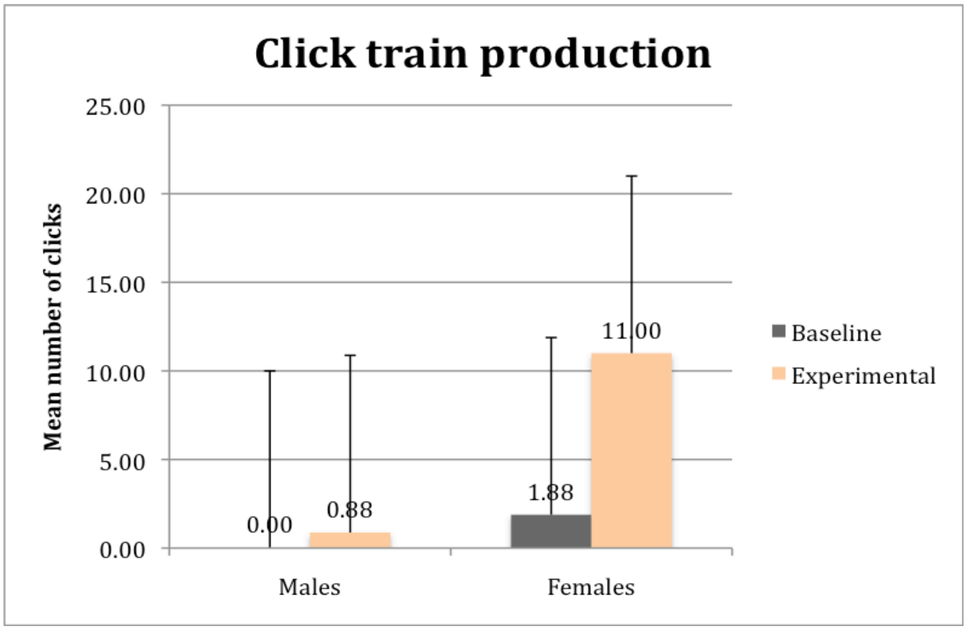


Figure 5.6. Mean number of click trains produced by male and female hippos during baseline (males n = 10, females n = 51) and experimental sessions (males n = 58, females n = 8).

Inspection of the frequency parameters of male and female click trains demonstrated similarities in the types of click trains produced during the sessions when carrots were offered. Thirty-five click trains produced by females were selected for comparison to the 35 produced by the two males. Only two click trains produced by the females had a mean peak frequency above 1000 Hz (broadband click train). The remaining 33 were narrowband click trains that were similar to the males with individual clicks that varied in bandwidth. The results of the autocorrelation of the combined male and female clicks trains (n = 66 narrowband, n = 9 broadband) demonstrated that all of the parameters were correlated, which prevented comparisons of the mean parameters of all the click trains. However, comparison of the parameters of the narrowband click trains for males versus females demonstrated that female clicks were slightly lower in frequency for the minimum, maximum, and peak frequencies (Table 13).

Table 13. Comparison of narrowband click trains (males n = 28, females n = 33)

	Minimum frequency (Hz)	**Maximum frequency (Hz)**	**Click duration (s)**	**Peak frequency (Hz)**	**Duration (s)**	**ICI (s)**
Male	352±83	2683±3106	0.017±0.005	654±102	0.659±0.702	0.059±0.018
Female	285±74	2514±2356	0.023±0.007	563±149	0.506±0.199	0.063±0.026

A logistic regression was used to examine whether certain environmental factors (increasing levels of turbidity (more or less than six feet), time of day (pre-dawn or post-dawn), carrot bundles pre-placed in the pool prior to the hippo entering or not, and whether the hippo was alone or not) or motivation influenced the occurrence of click trains. None of these conditions were significant predictors of click train occurrence (Table 14).

Table 14. Results from a logistic regression testing environmental conditions as predictors of click train occurrence.

	B	**S.E.**	**Wald**	**Df**	**Sig.**	**Exp(B)**
turbidity	19.77	9818.83	0.000	1	0.998	3.856E8
light	-39.87	14270.45	0.000	1	0.998	0.000
carrots	0.693	1.87	0.137	1	0.711	2.000
alone or not	0.739	22366.90	0.000	1	1.000	2.095

Discussion

The results of this study provide the first evidence that hippo click trains appear to serve a non-social function and may be used for echo-ranging. First, click trains were produced when the hippos were solitary suggesting they were non-social in function. Two solitary male hippos produced 51 click trains while searching for carrots when alone (n = 29 click trains) and when another hippo was in the pool (n = 22 click trains). No click trains were recorded from these hippos during baseline sessions or when hippos were not searching for carrots. If click trains function only as social signals, it would be expected that hippos would not have produced these signals while alone. In addition, there should have been an increase in click train production when more than one male hippo was present, but neither was the case.

Second, the occurrence of click trains correlated with search behavior that culminated in obtaining carrots. A majority of the click trains (82%) were produced less than a one-minute interval (n = 28 click trains) or between a one and five-minute interval (n = 14 click trains) prior to the hippo chewing underwater or observed chewing on the surface. Only nine click trains were produced after a five minute interval, suggesting clicks may function as echo-ranging signals (Figure 5.3). The increase in the time it took Hans to obtain carrot bundles in 2009 may be due to the sessions in which carrots were pre-placed in the exhibit pool prior to the hippo entering, which required more extensive searching.

Across the experimental sessions for each recording season, there was an increase in the occurrence of click trains (Figure 5.2). Although four male hippos were first conditioned in the barns to expect the delivery of carrots following the tone, it seems that the pre-training and experimental sessions conducted in their outdoor exhibit pools had a stronger effect on their response to the tone and carrots being tossed into the pool. Biko was only tested in the exhibit pool, which could explain the increase in his response across the sessions. While Hans may have learned to search for the carrots, he did not produce clicks in response to the tone or splash since he continued to produce click trains during the sessions when the carrots were pre-placed in the pool (Figure 5.2). During these sessions, the tone was played once prior to the hippo entering the pool and there were no splashes to cue the hippos of the location of the carrot bundles. However, the researcher’s presence may have been an additional cue that carrots were present in the pool.

Third, the acoustic structure of click trains matched the requisite frequencies to detect the size of carrot bundles used in this experiment. Our analysis of the acoustic structure of the click trains supported previous findings by Barklow (1997, 2004) that suggested two main types of clicks: narrowband and broadband (wideband) signals (Figure 5.4 and Table 12). Although individual clicks varied in bandwidth, the maximum and peak frequencies of the click train types were significantly different with narrowband clicks being lower in frequency (Table 12). To further examine the possible function of click trains, the optimal frequency for reflecting sound off of a spherical object was estimated using the formula $f = c/2\pi r$ (c is the speed of sound (1497 m/s underwater)) to determine whether hippo click trains could be used to detect the carrot bundles (Tyack, 1998). Although the carrot bundles were not spherical, half the length of the carrot bundles (5.5 inches or 0.14 meters) and half the width of the carrot bundles (2.5 inches or 0.063 m) to approximate an optimal detection frequency of 1702 Hz (based on length of carrot bundle) and 3752 Hz (based on the width of the carrot bundle). These optimal frequency calculations were higher than the mean peak frequency of both types of click trains, but within the frequency range of the click trains. The optimal detection frequency based on the length of the carrot bundles (1702 Hz) was close to the peak frequency of 1599 Hz for the broadband click trains, suggesting hippo clicks could be used to detect the carrot bundles (Table 12).

Male and female hippos produced both broadband and narrowband click trains that were similar in structure. The clicks produced by females were lower in frequency than the males. However, it is uncertain as to whether this is a difference in sex or age since the two males were younger (ages 5-7) than the females. It is also possible that there may have been some differences in the conditions in which carrot bundles were offered to the females, but further tests are needed to determine whether differences may exist (Table 13).

Hippos may use low frequency click trains to locate objects larger than the food items used in this experiment such as other hippos or for general navigation and orientation in murky environments. In addition, hippos, like dolphins, may be capable of controlling the amplitude and frequency modulation of clicks, along with the click train length, which may be dependent on the animal and/or the task (Houser et al., 1999). The click trains of dolphins show in decease in interclick intervals (ICI) as they hone in on a target (Au & Hastings, 2008); however, there was no significant change in the ICI of clicks trains produced by hippos.

Instead, the acoustic parameters of hippo click trains appear to resemble echolocation clicks produced by very young dolphins. Studies investigating the ontogeny of echolocation in dolphins have demonstrated that young dolphins first emit clicks around two weeks after birth that are longer in duration and lower in frequency (peak frequency below 4 kHz) than older dolphins (Reiss, 1988). These clicks are produced with their mouths open and correspond with increased exploration of their surroundings. By week three, young dolphins click with their heads oriented towards objects. As the calves mature, the clicks become shorter, higher in frequency and include changes in repetition rates, resembling adult clicks, and they no longer keep their mouths open (Reiss, 1988). Another study documented the ontogeny of a Yangtze finless porpoise calf that first emitted echolocation clicks concurrent with head scanning movements around postnatal day 22 that were indistinguishable from adults (Li et al., 2007). The researchers reported a decrease in the duration, maximum click repetition rate, and number of clicks per train as the calf matured. The initial production of clicks by young dolphins may be related to highly emotional or energetic contexts (Reiss, 1988), but they could still provide general information about their surroundings.

The ontogeny of echolocation in these cetacean species suggests that the clicks produced by very young dolphins are more rudimentary because their sensory systems are still developing. Like young dolphins, young bats explore their environment and produce echolocation signals that are longer and lower in frequency with their mouths open. Like dolphins, as bats mature, their signals increase in frequency, become shorter, and increase in repetition rate. For some bat families (Hipposideridae and Rhinolophidae) there is another development shift: young bats first emit echolocation signals from their mouths but later emit these signals from the nasal system (Brown & Grinell, 1980). While young dolphins first emit clicks with their mouths open (Reiss, 1988), older dolphins produce clicks from their nasal system with their mouths closed, which involves the nasal plug and two air sacs (Cranford et al., 1996). Interestingly, hippos have also been reported to produce click trains with their mouths open (Kreuger, 2000), although the mechanisms for sound production have not been studied. Hippo click trains were produced only underwater and on a few occasions included the expiration of a few bubbles; however, I could not determine whether their mouths were open while clicking or the source of the bubbles.

Although click trains were not produced during every experimental session, several factors may have influenced whether hippos produced click trains. It was predicted that increased levels of turbidity, less ambient light, and removal of the splash by placing carrot bundles in the pool prior to the start of the session would result in an increased occurrence of clicks trains. I also examined whether the hippo was alone or not to determine whether this condition may have influenced the occurrence of click trains. The results of the logistic regression did not support these hypotheses (Table 14), yet they warrant further considerations as possible factors influencing click train production. The river pool was murkier, especially once the hippo entered the pool and stirred up debris on the bottom, which may resemble conditions in rivers and lakes in Africa. In 2009, water quality had improved which is why more sessions were attempted in the pre-dawn hours. Fewer click trains may have been produced by one of the males in the underwater viewing pool because it was clear to the bottom and some lights could not be turned off. Sessions were attempted in the dark, but hippos were more likely to enter the pools and cooperate with the experiment when it was light out; only one click train was produced in the dark. However, I could not rule out the possibility that hippos could detect the carrots either by sight or smell since virtually nothing is known about hippo sensory systems. These environmental conditions should be evaluated in future studies.

With the exception of the night session during the pilot study, the male hippos only produced click trains during the experimental sessions when searching for carrot bundles. Female hippos produced click trains and other pulsed signals during the baseline sessions and during the eight sessions with carrots. The mean number of clicks produced by females increased from 1.88 during baseline sessions to 11 during the sessions when carrots were offered (Figure 5.6). This significant increase in click production suggested that females, like the males, could be using click trains for echo-ranging. However, due to the murky conditions and number of females in the pool, it was not possible to determine when the hippos were searching and obtaining carrots. Thus, it was also possible that click trains were associated with social interactions due to competition over the carrots. However, the female hippos may have used click trains to detect the location of conspecifics as well as the carrots, but further research is needed to determine the function of clicks.

Dolphins and several other cetaceans use a variety of pulsed signals for social communication (Au & Hastings 2008), foraging (e.g. Stimpert et al. 2009), and other contexts such as single clicks associated with fright (Caldwell et al., 1962), as well as click trains for echolocation. Other studies have suggested that echolocation clicks may serve a dual function for echolocation and communication (e.g., Dawson, 1991). For example, a recent study by Voigt-Heucke and colleagues (2010) provided evidence that lesser bulldog bats demonstrate social behaviors in response to echolocation calls from familiar versus unfamiliar conspecifics based on significant individual differences in the calls. Thus, it is possible that some pulsed signals produced by hippos may serve as social signals and others for echo-ranging, but the functional significance of these signals remains unclear.

Although wild hippos generally produce acoustic signals under higher excitement prior to moving on land at night to graze (Barklow, 1994, 1997, 2004; Karstad & Hudson, 1986; Walther, 1984), it does not appear that click trains in this study were associated with the anticipation of food. The hippos did not produce clicks in-air or prior to carrot bundles being offered. Click trains were only produced while hippos were searching underwater, and Hans continued to search for carrots when they were pre-placed in the pools. Besides the single night session during the pilot study, all sessions with males and females were conducted in the early morning, after the hippos had been fed in the barns, and when they first came into the pools.

Another possible explanation was that hippos used click trains to alert conspecifics to the presence and location of food, similar to what has been observed in the food calls of other species such as chickadees (e.g., Mahurin & Freeberg, 2009). Transient killer whales produced pulsed calls after killing prey, which may represent a food call, but more likely represents increased vocal activity due to increased social activity and/or a reduction in the potential for eavesdropping of prey during hunting (Deeke et al., 2005). Although the timing between click production and success in obtaining carrots could not be confirmed for the females, male hippos were mostly solitary and clicks were produced prior to obtaining carrots, as opposed to after finding food, suggesting clicks were not food calls. Further studies are necessary to compare the parameters and to test for the functional use of click trains to determine whether hippos evolved an echo-ranging system as they adapted to aquatic living.

Limitations

The results from this study demonstrate hippo click trains may be used for echo-ranging, but there are some limitations. First, all of the experimental sessions in this study were conducted with male hippos in the early morning hours between 5:30 AM when it was dark out to 8:30 AM when the sun had risen. In addition, the hippos did not always remain in the pools as they often went back on land to forage or dung shower, which limited the amount of time they participated in experimental trials. Except for the single night session of the pilot study, the hippos could not be tested at night due to scheduling restrictions. Sessions were attempted in the pre-dawn hours, but it was difficult to get the hippos to come out in the dark and there were exhibit lights surrounding the underwater viewing pool where Biko was tested that could not be turned off. Both hippos were more likely to enter the pools in the daytime because this was more consistent with their daily routines, which may also simulate their behavior in the wild. Sessions in the dark were attempted to prevent visual detection of carrot bundles, but hippos may have been using other senses to search for carrots underwater.

Although several males were trained on the object detection task, only two males successfully continued to search and obtain carrots. The other three males did not respond to the object detection task in the exhibit pools. Differences in motivation may be explained by changes in their daily routine or the shifting of other hippos, which often instigated dominance interactions between the males. For example, one explanation for the delay in Hans entering the pool or coming over to participate in the experiment was due to his waiting around the barn for other hippos to pass by and/or because he waited for Henry to come out since they were normally housed together during the day. The normal routine for two of the males' (Padron and Uzazi) was to stay in the river pools at night. Thus, their lack of cooperation in the morning may have been due to their anticipation of going in to the barn, which is also where they received food in the morning. One of the males (Uzazi) appeared to demonstrate patterned swimming in the pool prior to being called into the barns. One of the zookeepers attempted to call Uzazi over in the morning, but he would not approach the middle of the pool and he did not search for carrots. The other male, Padron, showed no interest in the carrots, but was motivated to search for watermelon. However, attempts to sink the watermelon were not successful.

Two of the males (Uzazi and Henry) were motivated to search for carrots in the barn and on land, but they did not search underwater in the exhibit pools. Although this difference may have been influenced by changes in their normal daily routines, it may have also been affected by the training procedures. Training first occurred in the barns but some hippos may not have transferred the association between the tone and carrots to the exhibit pools. In addition, there were some training differences depending on which staff members were training the hippos. It was discovered later that some only played the tone once and offered carrots, while other staff presented the tone and carrots multiple times during a session. This difference in training could also account for the increase in searching behavior across the experimental sessions.

Last, the carrots were tied together using corn husks or banana leaves to create a bundle sufficient in size to be detectable by the hippos, yet the bundles did not always stay together when they were tossed in the pool. As a result, the hippos may have been challenged to detect the single carrots. However, hippos could still locate individual carrots as they were observed searching for and chewing carrots even when the bundles broke apart.

Further research

Further studies investigating the function of hippo click trains could include more controlled conditions to test solitary male and female hippos at night or in murky water using larger objects that match the optimal frequency range of the narrowband click trains. Masking studies such as using eyecups may be used to rule out the possibility that other sensory systems may be used to detect objects. Additional studies should include more behavioral evidence for echo-ranging, including visual observations to determine when hippos are searching, whether their mouths are open, and whether bubbles are emitted during click train production. Further research is necessary to determine whether the acoustic properties of hippo clicks could yield echoes useful for obtaining information about their surroundings. Other parameters such as amplitude and external variables such as light levels and water clarity should be included to further examine the echo-ranging hypothesis in hippos.

Conclusion

This study provides the first evidence that hippo click trains serve a non-social function and may be used as echo-ranging signals. Three lines of evidence support this hypothesis. First, male hippos produced clicks trains in a non-social context while searching for carrot bundles. Second, most of the click trains were produced prior to obtaining carrots. Third, the frequency parameters of clicks could be used to detect the carrot bundles. Future studies of click train use in the field and captivity will be critical to further elucidate the sensory capabilities of hippos and the function of these signals. These findings will continue to reveal more information about how hippos have adapted to aquatic living and provide insights as to whether echo-ranging was inherited from the common ancestor of hippos and cetaceans or the result of convergent adaptations to life in the water.

Conclusions

The results of this study have provided important insights about the evolution of the hippopotamus and their adaptations to aquatic living. The review of literature on the evolution of hippos and their anatomy and morphology suggest hippos may have more adaptations for aquatic living than previously thought and share several adaptations with their close aquatic relatives, the cetaceans. Through a collaborative study, we found that the vocal folds of hippos are similar in orientation and structure to baleen whales, but further dissections are needed to examine their oral and nasal cavities and the mechanisms of sound production. However, it is not clear which adaptations represent convergent evolution or shared traits due to common ancestry.

My findings on hippo communication confirm previous descriptions of basic categories of acoustic signals used by captive and wild hippos, but my findings provide more details about the different categories and signal types. Male and female hippos produced most of their signals underwater and the majority of these were tonal signals. In addition to the tonal and pulsed categories described in previous studies (Barklow, 1997, 2004), a third category was added that represented burst of air signals. New signal types (e.g., chuffs, groans) were identified and eleven signal types were differentiated based on their acoustic parameters and behavioral contexts. Whines, croaks and clicks produced by these captive hippos were similar to those produced by wild hippos, but there were some differences in the behavioral contexts. The findings of this study suggest acoustic signals mediate social interactions since the signals were associated with dominance and submissive contexts such as appeasement (e.g., chuffs, whines), low-level aggression (e.g., snorts, grunts), and high-level aggression (e.g., screams). Wheeze honks were the most common signal produced by males and females and were identified as contact calls that were often produced as a chorus by several individuals. Wheeze honks produced by females were lower in frequency than those produced by males, but age should be taken into consideration for further comparisons. Females produced higher numbers of different signal types, but this may have been due to differences in group size.

Last, this research demonstrates the first evidence that hippo clicks may be used for echo-ranging. Click trains were produced by two male hippos in a non-social context when they were searching for and obtaining carrot bundles underwater. No click trains were produced by the males during baseline recordings or outside the search context of the experimental sessions. The frequency characteristics of the click trains matches the frequencies necessary to detect the carrot bundles used in this study and resemble the echolocation clicks of young dolphins. Females in social contexts produced click trains during baseline sessions, but increased the number of clicks produced when carrots were offered, suggesting they may also using click trains for echo-ranging. These results support the echo-ranging hypothesis in hippos since both males and females produced click trains more frequently when they were searching for carrots underwater, suggesting they may use clicks to detect objects in the murky waters they inhabit.

This research provides new information about the communication and sensory systems of hippos. Additional research is needed to further investigate their anatomy, sensory systems, and other modalities for communication. A better understanding of the amphibious lifestyle and behavior of hippos will ultimately improve the management and protection of this species in captivity and the wild. Research on hippos has become increasingly important as they are currently listed as Vulnerable on the IUCN's red list (Kanga et al., 2011). Hippos face increasing threats of habitat loss and poaching for the ivory in their canines. Due to their territorial and aggressive nature, hippos have frequent conflicts with humans and are considered a threat to the human populations near water sources (Feldhake, 2005). Over the years, hippo populations have been culled to prevent these conflicts and to reduce the damage they cause to grazing land. However, hippos play a critical role in the rivers and lakes they inhabit, providing food for fish as they re-enter the water after grazing at night that is often understated.

These findings will hopefully encourage other researchers to conduct further studies of the anatomy, behavior, and communication of hippos to reveal more information about how they have adapted to their amphibious lifestyle and their evolutionary connections to cetaceans.

Appendix

Ethogram of hippopotamus (*Hippopotamus amphibius*) behavior (adapted from Blowers et al., 2009)

Behavior	**Description**
Foraging	Animal consuming produce, plant material, or hay
Dung shower	Animal wags tail and spreads dung and urine onto nearby land or walls
Inactive	Animal not moving
Locomotion	Animal is walking around in or out of water or is porpoising (locomoting up, out, and back into water)
Social behaviors	
Alert	Animal raises head above the surface of the water (scans?)
Approach (and contact)	One animal moves toward another animal within one body length (note if physical contact is made between animals)
Rafting, no head resting	Animals are together in bundle with no head resting activity (note which animal the focal animal is next to)
Rafting, head resting	One animal has chin propped onto the back or rear of another animal (note the identity of head rester and identity of animal rested on)
Face to face	Two animals are face to face with each other with no other movement
Follow	One animal follows another for a period of time longer than 10 seconds
Head rest avoid	One animal moves to not allow another animal to rest its head on the first animal (note which animal was denied access to head rest)
*Agonistic (*low level aggression, **high level aggression)*	
Charging**	One animal lunges toward another animal within one body length (note if physical contact is made between animals)
Head shaking*	One animal shakes head vigorously from side to side (note which animal it is directed toward)
Head slap*	Animal slaps head on water with mouth closed
Head thrusting*	One animal thrusts head upward toward another animal (note which animal it is directed toward)
Jaw clashing**	Jaw-to-jaw striking while moving the head from side to side or forward and backward with physical contact between two animals (note which animal retreats from sparring first)
Jaw slap*	Animal opens and closes mouth while slapping the bottom part of jaw vigorously on the surface of the water
Open mouth*	Animal opens its mouth and exposes tusks
Open mouth scoop*	Animal opens its mouth and exposes tusks and then scoops down and back out of the water
Tusk slashing**	One animal slashes at the body of another animal with its tusks
Submissive	
Away	Animal moves away from conspecific
Crouch	One animal lowers the legs, placing its body on the ground in response to another animal
Rear turn	One animal turns its rear toward approaching animal
Side turn	One animal turns its side toward approaching animal
Tail paddling	Animal moves the tail up and down at the surface of the water, may include dung shower
Other	
Underwater bubbles	Animal releases air underwater
Other behavior	Any other behavior not listed
Out of view	Animal is physically out of viewing sight
Not visible behavior	Animal can physically be seen but behavior of animal is not visible
Vocalizations	
Snorting	Animal snorts (blow air out of nostrils)

Snorting/Repetitive	Focal animal breathes rapidly, repetitively, and forcefully through nostrils, for 2 or more breaths, directed at a conspecific
Wheeze-honk	Animal wheezes followed by several honks
Other vocalizations	Any vocals other than snorting, underwater bubbles, or wheeze-honk
Head position	
Above	Head completely above the surface
Nostrils beneath the surface	Eyes and ears still above the surface
Nostrils above the surface	Amphibious position, eyes, ears and nostrils above the surface, mouth, throat and body below

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