

**Direct and Indirect Effects of Recreational Boats
on Diamondback Terrapins (*Malaclemys terrapin*)**

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Lori A. Lester

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ABSTRACT

Direct and Indirect Effects of Recreational Boats
on Diamondback Terrapins (*Malaclemys terrapin*)

Lori A. Lester

Advisor: Dr. Harold W. Avery

Recreational boating has become an increasingly popular activity over the past 100 years as a result of extensive human population growth in coastal areas. Recreational boats may affect aquatic organisms directly through injury and mortality due to boat strikes and indirectly through increased levels of anthropogenic sounds. The objective of this study was to determine whether recreational boats affect diamondback terrapin (*Malaclemys terrapin*) populations in Barnegat Bay, New Jersey, USA. I used six years of mark-recapture data to determine whether terrapin injury rates have increased temporally and whether larger terrapins had a higher risk of anthropogenic injury than smaller terrapins. I also used a Cormack-Jolly-Seber (CJS) model to determine if survivorship differed between injured terrapins and uninjured terrapins. In some locations, injury rate of terrapins increased from 2006 to 2011. Larger terrapins were found to be more likely than smaller terrapins to be injured by boats or automobiles. Injured terrapins in some locations also had significantly lower survivorship than uninjured terrapins. I recorded Auditory Evoked Potentials (AEPs) to determine hearing capability of diamondback terrapins in air and underwater. Terrapins responded to sounds from 100 to 1000 Hz with best hearing from 400 to 600 Hz in air. Underwater, terrapins responded to sounds from 50 to 800 Hz with mean lowest threshold of 86 dB re 1 μ Pa rms. I recorded sounds of recreational boats and personal watercraft (PWC) in Barnegat Bay and found that boats and PWCs produce sounds that are in the hearing range of

diamondback terrapins. Diamondback terrapins were exposed to playback recordings of approaching boat engines *in situ*. I measured behavioral responses to boat engine sounds including swimming speed, swimming depth, and orientation of terrapin in the water column. Diamondback terrapins did not behaviorally respond to sounds of approaching boats. The lack of behavioral response of diamondback terrapins to recreational boats threatens the survival of terrapin populations. Conservation actions (such as boater education, speed limits, and partial or complete closures of wildlife areas to boats) must be initiated to protect aquatic wildlife from injury and mortality from recreational boats.

CHAPTER 1: INTRODUCTION

Over half of the world's human population lives in coastal areas and these coastal populations continue to increase in size (Vitousek et al. 1997). As a consequence of high human population on coasts, human activities such as recreation and tourism have also increased drastically in these areas. In particular, recreational boating in the USA has increased temporally since the end of World War II (Anderson 1996).

Recreational boats may affect aquatic animal populations directly and indirectly. The direct effects of recreational boats on aquatic animals include injury and mortality resulting from impacts from boat propellers or hulls (Ackerman et al. 1995, Bulte et al. 2010). Many animal populations, such as manatees (*Trichechus manatus*), crocodiles (*Crocodylus niloticus*), and loggerhead sea turtles (*Caretta caretta*), are negatively affected by boat strikes (Ackerman et al. 1995, Oros et al. 2005, Grant and Lewis 2010).

Recreational boats may indirectly affect an aquatic species through anthropogenic (human-generated) sounds produced by boat engines. Anthropogenic sounds may affect aquatic animals in several ways (Richardson et al. 1995). First, the animal may alter its behavior. For example, sperm and pilot whales stop vocalizing during exposure to anthropogenic sounds (Bowles et al. 1994). Second, aquatic animals may be prevented from hearing important natural sounds because anthropogenic sounds are more intense. The sound created by icebreakers can mask the call of beluga whales (Erbe and Farmer 1998). Third, the animal may experience temporary or permanent hearing loss. Researchers exposed bottlenose dolphins and beluga whales to high intensity pure tones and found that these sounds caused temporary threshold shift (TTS) which is a temporary form of hearing

loss (Schlundt et al. 2000). Fourth, the animal may experience tissue damage. McCauley et al. (2003) found that exposure to the sounds of air guns used in marine petroleum exploration causes extensive ear damage in fish. The main goal of this dissertation research was to determine the direct and indirect effects of recreational boats on diamondback terrapins (*Malaclemys terrapin*) in Barnegat Bay, New Jersey, USA.

Background on Diamondback Terrapins

Diamondback terrapins are an excellent model organism for several reasons. Diamondback terrapins are an amphibious species: they forage and mate in the water and bask and nest on land. Thus terrapins can be studied in both terrestrial and aquatic habitats. Furthermore, terrapins are a relatively long-lived species with a lifespan of 30 or more years which allows the population to be studied for an extended period of time (Roosenburg and Kelly 1990). They are also relatively easy to capture and handle. Terrapins have a hard shell which may remain after the individual has died, allowing data to be collected from an individual regarding possible mortality source. Diamondback terrapins exerts top-down control on populations of periwinkle snails (*Littoraria irrorata*) which graze on salt marsh cordgrasses (Silliman and Bertness 2002) suggesting that terrapins are a keystone species and thus crucial to maintaining diversity in estuarine ecosystems.

Diamondback terrapins are the only North American turtle species to reside solely in brackish water habitats such as salt marshes, estuaries, open bays, and mangroves. Seven subspecies of terrapins extend along the East and Gulf coasts of the United States from Cape Cod, MA to Corpus Christi, TX (Brennessel 2006). The species that is found in New Jersey is the Northern Diamondback Terrapin (*Malaclemys terrapin terrapin*) and

they are listed as a species of special concern by the state (Watters 2004). Adult terrapins are sexually dimorphic in respect to their size, thus females are typically more than twice the size of males (Gibbons and Lovich 1990). Diamondback terrapins are active in warm months from approximately April to October or November depending on where they are located in the terrapin range. Females nest from the end of May to the middle of July and can lay multiple clutches in one year. In cooler months and parts of the range, the turtles will brumate (i.e., a dormancy period in reptiles similar to hibernation) in the bottom sediments and respire through cloacal respiration (Brennessel 2006).

Terrapin populations were severely depleted in the late 1800s and early 1900s due to the human consumption (Carr 1952). Terrapin meat was considered a gourmet delicacy, most commonly served in turtle stew. During prohibition, terrapin populations rebounded slightly because sherry was a key ingredient in turtle stew. Today, terrapin populations are still regarded as no listing, game species, endangered, threatened, or species of special concern as determined by the state where the population is located (Watters 2004). In addition to exploitation as a food source, other anthropogenic threats are causing diamondback terrapin population declines. Habitat destruction has led to a loss of nesting locations and also increased mortalities due to collisions between nesting female terrapins and automobiles (Wood and Herlands 1997, Szerlag and McRobert 2006, Szerlag-Egger and McRobert 2007). Juvenile and adult terrapins are incidentally captured in crab traps and drown (Bishop 1983, Roosenburg et al. 1997, Wood 1997, Tucker et al. 2001). Furthermore, terrapins are often injured (sometimes to the point of mortality) by non-native predators (Draud et al. 2004) and recreational boats (Cecala et al. 2009). These types of anthropogenic injuries may lead to difficulties with foraging, avoiding predators,

and reproducing for individual terrapins and eventually to population declines (Werner and Anholt 1993).

Barnegat Bay, New Jersey

Barnegat Bay is a 70 km length estuary located along the central coast of New Jersey (BBNEP 2002). In this watershed, 600,000 people live year round and that number almost doubles due to tourists during the summers (BBP 2011). This region has been experiencing changes due to anthropogenic disturbances for at least 350 years beginning when the Europeans settled in the middle of the 17th century (BBNEP 2002). Many environmental concerns for the estuary exist including nutrient overload, pollutants, pathogens, human population growth, habitat degradation, species decline, fisheries loss, and introduced species (Kennish and Lutz 1984).

Estuaries are biologically important because they are one of the most productive ecosystems on the planet and are critical habitats for various wildlife species (McLusky and Elliot 2004). Furthermore, estuaries serve as nursery grounds or migratory pathways for some oceanic species. Estuaries are also economically important to humans because they are popular locations for not only human settlement but also activities such as fishing and recreation. These biological and economic functions of estuaries oftentimes overlap and lead to issues due to anthropogenic disturbances such as overfishing, habitat destruction, and pollution.

Minimal research has been completed on the effects of anthropogenic sounds on wildlife species in estuarine environments. In the Peconic Bay Estuary in Long Island, New York, average sound pressure level (SPL) during peak human activity was 110 dB, much of which were low-frequency sounds created by recreational boats (Samuel et al.

2005). Anthropogenic SPLs from recreational boats and effects of these human-generated sounds on estuarine species have not been evaluated in Barnegat Bay, New Jersey.

Turtle Injury Rates

Turtles often sustain major and minor injuries from natural and anthropogenic sources (Lovich and Gibbons 1990, Roosenburg 1991, Burger and Garber 1995, Hart and McIvor 2008, Cecala et al. 2009). The most common natural injury source is native predators. Anthropogenic injuries are often due to automobiles, non-native predators, and boat strikes. Recreational boats have both direct and indirect impacts on various aquatic organisms including turtles (Bulte et al. 2010). The direct effects include injuries or mortality from collisions with boat propellers or hulls (Gibbons et al. 2001, Cecala et al. 2009). Indirect impacts of power boats have less obvious effects on aquatic animals. In response to recreational boats, yellow-blotched map turtles (*Graptemys flavimaculata*) reduce nesting rates, sperm whales (*Physeter macrocephalus*) decrease vocalizations, largemouth bass (*Micropterus salmoides*) experience increased heart rates, and fathead minnows (*Pimephales promelas*) have decreased hearing sensitivity (Bowles et al. 1994, Scholik and Yan 2002, Moore and Seigel 2006, Graham and Cooke 2008).

Oftentimes, it is difficult to determine if an animal was injured by a natural or anthropogenic source unless an observer is present but inferences about injury source can be made in certain instances. For example, in Kiawah Island, South Carolina, 12% of females and 8% of males are missing one or more limbs (Lovich and Gibbons 1990). These limb injuries were attributed to predation due to an overabundance of terrestrial predators such as raccoons in this area. More recently in Kiawah Island, 10.8% of captured terrapins had a major injury with 8% missing a limb and 2.8% having major shell damage

(Cecala et al. 2009). In the Everglades National Park, FL, 16% of terrapins exhibit injuries such as missing limbs, carapace damage, and tail damage (Hart and McIvor 2008). Major carapace and/or plastron damage is often assumed to have been caused by anthropogenic sources such as automobiles and boats. Oftentimes, a slash mark can be seen in the carapace and/or the plastron from a boat propeller. In North Carolina, 6% of the terrapin population sustained injuries (Hart, personal communication). Furthermore, 19.7% of female terrapins and 2.2% of male terrapins in the Chesapeake have scars from boat propellers (Roosenburg 1991). When diamondback terrapins are struck by boat propellers, this may result in instant death where many of these individuals are not recovered (Crowder et al. 1995). Moreover, injured terrapins must survive to next capture in order to be counted towards injury rate calculations. Thus calculated injury rates are likely underestimates of the actual proportion of terrapins that are injured or killed.

Aerial and Underwater Hearing in Turtles

The sense of hearing is essential for many aquatic organisms because visual cues are often limited due to turbidity and lack of light. Two theories exist to explain the importance and evolution of the sense of hearing in vertebrates (Fay and Popper 2000). The first theory is that the auditory system evolved as part of an acoustic communication system (Hauser 1997). Many vertebrate species acoustically communicate with other conspecifics. Even some turtle species, such as the long-necked freshwater turtle (*Chelodina oblonga*), have been found to vocalize (Giles et al. 2009). The second theory is Auditory Scene Analysis (ASA). ASA is the concept that the listener can determine individual sources from multiple simultaneous sources (Hartman 1988, Yost 1991). These

components are used by listeners to develop an auditory image of their omnidirectional surroundings whereas vision only allows knowledge of one direction.

Turtles do not have an external ear and rarely vocalize, thus it was previously assumed that they cannot hear (Wever 1978, Lenhardt 1981). Behavioral and physiological research has demonstrated that various freshwater and marine turtle species can hear sounds under 1000 Hz (Ridgway et al. 1969, Wever 1978, Corwin et al. 1982, Bartol et al. 1999, Bartol and Ketten 2006, Heffner and Heffner 2007).

Morphological studies on freshwater turtle species, such as red-eared sliders (*Trachemys scripta elegans*), have found that although the external ear is absent, there are middle and inner ear structures present under the tympanic membrane (Wever 1978, Lenhardt and Harkins 1983). The middle ear is air filled and has an ossicular chain consisting of an extracolumella directly beneath the tympanum and a columella (Saunders et al. 2000). The columella leads into a cone-shaped stapes that extends to the oval window of the cochlea (Wever 1978). The stapes and oval window are connected to the saccule by stapedosaccular strands which are unique to turtles. The perocapsular recess is fluid-filled and surrounds the otic capsule. A fluid circuit exists that extends from the inner surface of the stapedial footplate through the pericapsular recess to the outer surface of the footplate. This fluid circuit is stimulated by movement of the stapes.

The turtle inner ear consists of the cochlear duct in the otic capsule and the basilar membrane (Wever 1978). The basilar membrane contains hair cells which convert mechanical sound waves to electrochemical signals that can be received by the auditory nerve. The position of each hair cell on the basilar membrane dictates its characteristic

frequency with hairs cells near the apical end detecting low frequencies and cells by the basal end detecting higher frequencies (Crawford and Fettiplace 1980).

Marine turtle ears have also been studied morphologically and have been found to be similar to the freshwater turtle ear (Lenhardt et al. 1985). The two major differences between sea turtle ears and freshwater turtle ears are: (1) air can be passed from the oral cavity to the middle ear in sea turtles, and (2) there is a thick fat body under the tympanic membrane in sea turtles.

Auditory Evoked Potentials (AEPs) have been recorded in some turtle species to determine hearing capability. The AEPs measure the neurons that are discharged in the auditory pathway when an auditory stimulus is presented (Bartol and Ketten 2006). Many marine turtle species (Ridgway et al. 1969, Bartol et al. 1999, Bartol and Ketten 2006) and freshwater turtle species (Christensen-Dalsgaard et al. 2012) respond physiologically to low frequency sounds. In 1969, Ridgway et al. measured cochlear response potentials of green sea turtles (*Chelonia mydas*) to aerial and vibrational sound stimuli between 50 and 2000 Hz. The practical range of hearing for green turtles is from 50 to 1000 Hz with maximum sensitivity between 300 and 400 Hz (Ridgway et al. 1969). Juvenile loggerhead sea turtles (*Caretta caretta*) respond to low frequency clicks and tone bursts in air from 250 Hz to 750 Hz (Bartol et al. 1999). However, juvenile loggerhead turtles may be able to detect frequencies lower than 250 Hz because loggerhead turtles were most sensitive to 250 Hz and lower frequencies were not tested. Kemp's ridley sea turtles (*Lepidochelys kempii*) respond to underwater sounds from 100 to 500 Hz with best sensitivity from 100 to 200 Hz (Bartol and Ketten 2006). Hatchling loggerhead turtles, juvenile loggerhead and green turtles, and sub-adult green and Kemp's ridley turtles respond to sounds from 100

Hz to a maximum of 900 Hz (Bartol and Ketten 2006). Red-eared slider turtles (*Trachemys scripta elegans*) also respond to sounds from 100 to 1000 Hz with best sensitivity from 200 to 500 Hz in air and 400 to 500 Hz in water (Christensen-Dalsgaard et al. 2012). To our knowledge, hearing capability of diamondback terrapins has not been determined prior to this dissertation.

Effects of Anthropogenic Sounds on Turtles

Several studies have focused on how turtles perceive and respond to human-generated sounds from aircrafts, seismic air guns, and recreational boats. Desert tortoises (*Gopherus agassizii*) respond to simulated subsonic and supersonic aircraft sounds by exhibiting alert, startle, run, or freeze behavior (Bowles et al. 1999). In the most extreme cases, tortoises will cease movement for over 100 minutes and average heart rate can decrease by 7-8%.

Low-frequency sounds at high intensity levels can be used to deter animals from entering a specific area which could be useful in preventing at-risk species from entering an unsafe coastal area or interacting with fishing gear. O'Hara and Wilcox studied whether seismic air guns could be used to prevent loggerhead turtles from entering a water intake canal for a nuclear power plant in Florida (1990). The turtles would not enter an area within a 30 m perimeter of the sound; however, the sound output was 200 dB re 1 μ Pa at 1 m in the 250 to 1000 Hz range to illicit this response. These sound levels may be misleading because the reflection of sound on the canal walls was not taken into account. Turtle behavioral responses were also erratic with some turtles remaining directly below the sound source during trials.

The possibility of using auditory deterrents to clear sea turtles from the paths of hopper dredges has also been studied (Moein et al. 1994). Juvenile loggerhead sea turtles were held in net enclosures in the York River in Virginia with an air gun at each end of the net. The turtles were observed for behavioral responses to air gun blasts from 100 to 1000 Hz at three decimal levels (175, 177, and 179 dB re 1 μ Pa at 1 m) every five seconds for five minutes. Turtles avoided the region where the air guns were located; however, the behavioral response declined as exposure to the sound was repeated. The decrease in avoidance was likely due to either habituation or temporary threshold shift.

Similar results were found regarding behavioral responses to seismic surveys of loggerhead and green sea turtles (McCauley et al. 2000). Turtles increased swimming behavior when exposed to air guns that were producing sounds above 166 dB re 1 μ Pa rms. Above 175 dB, sea turtle behavior became erratic suggesting that they were agitated. Only two sea turtles were tested thus these results may not be an accurate representation of behavioral responses of sea turtles to seismic surveys.

Complications can arise when sound is used to deter animals from a certain area or situation. High frequency pingers have been used to prevent marine mammals from approaching gillnets (Southwood et al. 2008). Unfortunately, the cetaceans become habituated to pingers and may not respond to common sounds. The animals may also begin to associate the sound with a food source due to the fish caught in gillnets and thus approach the pinger to feed instead of avoiding the fishing gear. Furthermore, the high intensity sounds necessary for deterring animals may affect other non-target species and prevent game fish from approaching the fishing gear.

Limited research has been conducted on the effects of recreational boat engine sounds on turtles. Captive loggerhead, Kemp's ridley, and green sea turtles were exposed to simulated sound signals representative of recreational boating activities (Samuel 2004). The simulated boat sounds had frequencies from 300 to 600 Hz with sound pressure levels between 110 and 120 dB re 1 μ Pa rms. In response to the boat sounds, turtles increased submergence time between breaths when exposed to higher intensity signals and spent more time underwater during sound exposure.

Research Questions and Dissertation Structure

Many estuarine habitats, including Barnegat Bay, New Jersey, are currently experiencing high levels of recreational boat use which may affect terrapin populations. The main goal of this dissertation was to determine if recreational boats directly and indirectly affect diamondback terrapins. Understanding the effects of recreational boats on diamondback terrapins is essential for developing management plans to sustain and potentially enhance terrapin populations. The major research questions presented in this dissertation are as follows:

1. Are diamondback terrapins directly affected by injury and mortality from recreational boats in Barnegat Bay, New Jersey, USA?
2. Can diamondback terrapins hear in air and underwater?
3. Do diamondback terrapins behaviorally respond to the sounds of approaching recreational boat engines?

The main objective of chapter two was to determine direct effects of recreational boats on diamondback terrapins in Barnegat Bay, NJ. As part of an ongoing mark-recapture study on the population ecology of terrapins in Edwin B. Forsythe Wildlife

Refuge and Island Beach State Park, terrapin injury rates (many of which were boat strikes) were calculated from 2006 to 2011. Chapters three and four focused on assessing the hearing capability of diamondback terrapins in air and underwater. AEPs were recorded and audiograms were created to show the frequency range and hearing threshold for diamondback terrapins. In chapter five, diamondback terrapins were exposed to playback recordings of approaching recreational boats *in situ*. Behavioral responses of terrapins were measured including swimming speed, swimming depth, and orientation in the water. Finally, chapter six provides a summary of the major findings of this dissertation, potential future direction for research, and management recommendations for diamondback terrapin conservation in Barnegat Bay, NJ and other North American estuaries.

CHAPTER 2: INJURY RATES AND SURVIVORSHIP OF DIAMONDBACK TERRAPINS EXPOSED TO RECREATIONAL BOATS IN THEIR NATURAL ENVIRONMENT

Abstract

As part of an ongoing long-term population assessment of a diamondback terrapin (*Malaclemys terrapin*) population in Barnegat Bay, New Jersey, we used six years of mark-recapture data to determine the effect of injuries on terrapins. We quantified the frequency of injuries over time including anthropogenic injuries from automobiles or recreational boats and missing limbs from predators. We also studied the relationship between frequency of injury and size of terrapins and calculated the body condition index (CI) for injured and uninjured terrapins. We also compared survivorship of injured and uninjured terrapins using program MARK. Frequency of anthropogenic injury increased temporally in one location. Larger terrapins had higher rates of injury than smaller terrapins. Terrapin mass was not significantly reduced due to the presence of an anthropogenic injury and CI did not differ between injured and uninjured terrapins. In some locations, female and male terrapins with anthropogenic injuries had significantly lower survivorship than uninjured terrapins. Management of coastal and estuarine ecosystems should include protected areas to reduce anthropogenic injury to wildlife. Further studies are necessary to determine what types of predators exist and whether eradication is necessary.

Introduction

Many aquatic organisms, including diamondback terrapins, are prone to injuries from natural and anthropogenic sources (Cecala et al. 2009). Injuries due to anthropogenic

sources can have direct (injury or mortality) and indirect (behavioral changes, interference with vocal communication, altered auditory sensitivity) impacts on aquatic organisms (Scholik and Yan 2002, Moore and Seigel 2006, Giles et al. 2009). Diamondback terrapin populations frequently are impacted by anthropogenic injuries. In the Big Sable Creek complex in Everglades National Park, FL, 16% of terrapins exhibit injuries such as missing limbs, carapace damage, and tail damage (Hart and McIvor 2008). In North Carolina, 6% of the terrapin population sustained injuries. In Kiawah Island, South Carolina, 12% of females and 8% of males were missing one or more limbs (Lovich and Gibbons 1990). In 2009 in Kiawah Island, 10.8% of captured terrapins had a major injury with 8% missing a limb and 2.8% having major shell damage (Cecala et al. 2009). Furthermore, 19.7% of female terrapins and 2.2% of male terrapins in the Chesapeake have shell scars from propeller strikes (Roosenburg 1991).

Since 2006, we have been conducting a population study of diamondback terrapins in Barnegat Bay, New Jersey. Our main objective was to determine if terrapins are impacted by injuries, particularly those caused by automobiles, recreational boats, and predators. Specifically, we addressed five questions: (1) does the frequency of injuries to terrapins increase temporally, (2) does the frequency of injury increase with terrapin size, (3) do injured terrapins experience reduced body condition in comparison to uninjured terrapins, (4) do body condition indices (CI) differ between uninjured and injured terrapins, and (5) is survivorship lower in terrapins with anthropogenic injuries? These analyses allowed us to make effective management and conservation suggestions towards protecting diamondback terrapins from anthropogenic injuries.

Materials and Methods

Study Area

We conducted our study in the Barnegat Division of Edwin B. Forsythe Wildlife Refuge (Forsythe) and in Island Beach State Park (IBSP) in Barnegat Bay, New Jersey, USA (Fig. 2-1). Two sites were located in IBSP: Sedge Island and Spizzle Creek. We did not directly measure recreational boating; however, the number of recreational motor boats registered in the USA has increased by 17% over the past ten years (MTA 2008).

Capture and Processing Methods

We carried out this injury rate analysis as part of an ongoing mark-recapture population assessment of the diamondback terrapins in Barnegat Bay, New Jersey. Our sampling efforts varied in Forsythe and IBSP, but we sampled all study sites during spring and summer months from May to September. We sampled Forsythe from 2006 to 2011, Sedge Island from 2006 to 2011 except for 2007, and Spizzle Creek from 2005 to 2011. We captured terrapins with hoop nets, fyke nets, dip nets, or by hand in Forsythe and Spizzle. Traps were baited with bunker (*Brevoortia tyrannus*), set in shallow water, and checked daily. Captured terrapins were taken to the lab to be processed and released the next day at the site of capture. We captured terrapins post nesting by hand on Sedge Island. All terrapins were marked by notching the marginal scutes of the carapace with four to six letter codes (Cagle 1939) and passive integrated transponders (PIT) were injected into adult female terrapins (Biomark). Terrapins were also measured, aged, and sexed. Sex was determined by length of carapace, thickness of tail, and position of cloacae (Tucker et al. 2001).

We collected data regarding terrapin injuries including presence or absence, location (carapace, plastron, limb, head, or tail), and description of injury. We defined major shell injuries using the same technique as Cecala et al. where major shell injuries included an injury to two or more adjacent vertebral, costal, or plastral scutes (Fig. 2-2), or three or more adjacent marginal scutes (Cecala et al. 2009). We classified all terrapins with major shell injuries as anthropogenic injuries due to recreational boats or automobiles. We also recorded missing limbs which could be due to predators or anthropogenic sources.

Data Analysis

We compared yearly injury frequencies for three different groups: all injuries, anthropogenic injuries, and missing limbs. The all injury category included anthropogenic injuries, missing limbs, and minor injuries to head, carapace, plastron, tail, or limb. Frequency of anthropogenic injuries was then compared with plastron length via linear regression to test the hypothesis that larger terrapins had a higher frequency of injury. Frequency of injury was calculated from the mean value of 5 mm intervals of plastron lengths for each individual's first capture. Plastron length and mass of terrapin were also compared to assess whether terrapins experienced reduced mass as a result of anthropogenic injuries. An ANCOVA determined if mass differed significantly between anthropogenic injured and uninjured terrapins. We also computed mean body condition index (CI) and compared it for anthropogenic injured and uninjured terrapins in each study site (Wallis et al. 1999). Body condition index was equal to the mass (g) of each terrapin divided by volume (straight carapace length X straight carapace width X carapace height). Significance was set at $\alpha = 0.05$ for all analyses.

We used an open population model, the Cormack-Jolly-Seber (CJS), in Program MARK to determine if survivorship varied between anthropogenic injured and uninjured terrapins in Forsythe Refuge, at Sedge Island, and in Spizzle Creek. The CJS model made four assumptions: (1) there was no change in recapture or survivorship probability between marked and unmarked individuals, (2) marks were permanent and not lost over time, (3) animals were released immediately after capture, and (4) emigration rate was constant. Forsythe and Sedge datasets fit the model assumptions because we sampled terrapins yearly, notch markings had no known negative consequences, terrapins were in captivity for a short period of time (less than 24 hours), and emigration rates were not known to change. Male and female terrapins were analyzed separately because terrapins are sexually dimorphic in size and causes of injuries may differ between sexes. We estimated survivorship between groups (g) and among years (t). Models assumed constant or variable survivorship (Φ) over time or between injured and uninjured terrapins and the probability of recapture (p) was assumed to vary temporally. Akaike's Information Criterion (AIC) was used to assess goodness of fit (White and Burnham 1999).

Results

In Forsythe, mean injury rate was 18%, mean anthropogenic injury rate was 11%, and mean missing limb rate was 5% from 2006 to 2011. Injury and anthropogenic injury rates increased temporally from 2006 to 2011; however, missing limb rate did not increase temporally (Table 2-1, Fig. 2-3). At Sedge Island, mean injury rate was 22%, mean anthropogenic injury rate was 15%, and mean missing limb rate was 1%. In Spizzle Creek, mean injury rate was 19%, mean anthropogenic injury rate was 13%, and mean missing

limb rate was 2%. In the two IBSP sites, injury rates did not significantly increase (Table 2-1).

Mean injury rate for female terrapins in Forsythe was 21% and mean injury rate for male terrapins was 15%. Female terrapins were more likely to have an injury in Forsythe than male terrapins ($\chi^2 = 15.9$, $df = 1$, $P = 0.00007$). In Spizzle Creek, mean injury rate for female terrapins was 21% and for male terrapins was 11%. Female terrapins in Spizzle Creek were more likely than male terrapins to have an injury ($\chi^2 = 6.9$, $df = 1$, $P = 0.009$). Only nesting female terrapins are collected on Sedge Island, thus sex differences in injury rates could not be evaluated at that location.

Larger terrapins were more likely to have anthropogenic injuries than small terrapins in Forsythe (females, $Y = 0.003 X - 0.26$, $R^2 = 0.75$, $P = 4.0 \times 10^{-6}$; males, $Y = 0.003 X - 0.25$, $R^2 = 0.63$, $P = 0.03$; Fig. 2-4). Larger female terrapins on Sedge Island were also more likely to have anthropogenic injuries than smaller terrapins ($Y = 0.005 X - 0.68$, $R^2 = 0.86$, $P = 2.9 \times 10^{-4}$). In Spizzle Creek, larger female ($Y = 0.001 X - 0.10$, $R^2 = 0.50$, $P = 0.02$) and male ($Y = 0.001 X - 0.002$, $R^2 = 0.65$, $P = 0.02$) terrapins were more likely to have anthropogenic injuries than smaller individuals.

Diamondback terrapins of either sex did not have reduced mass as a result of anthropogenic injuries (Table 2-2). There was no significant difference between mass of injured and uninjured terrapins in Forsythe (ANCOVA, Females, $F = 2.69$, $df = 1$, $P = 0.10$; Males, $F = 0.33$, $df = 1$, $P = 0.57$). Female terrapins in IBSP also did not have reduced mass as a result of anthropogenic injury in Sedge ($F = 0.02$, $df = 244$, $P = 0.88$) or Spizzle ($F = 0.21$, $df = 374$, $P = 0.65$; Table 2-2). Injured terrapins did not have

significantly different body condition indices than uninjured terrapins in any of the study sites (Table 2-3).

The CJS model that best fit our data assumed constant annual survivorship over time with differences between terrapins with anthropogenic injuries and uninjured terrapins. Male terrapins with anthropogenic injuries in Forsythe had lower survivorship (Student's T-test; $P = 0.02$) than uninjured terrapins (Table 2-4; Fig. 2-5). Injured female terrapins in Forsythe did not have lower survivorship than uninjured female terrapins ($P = 0.32$). Anthropogenic injured female terrapins in Sedge ($P = 0.04$) and Spizzle ($P = 0.008$) had significantly lower survivorship than uninjured terrapins in the same location. Male sample size was too small on Sedge Island and in Spizzle Creek to complete the analysis.

Discussion

Diamondback terrapins in Barnegat Bay exhibited injuries at a higher rate than terrapins in most parts of their range with the exception of the Chesapeake Bay (Lovich and Gibbons 1990, Roosenburg 1991, Hart and McIvor 2008, Cecala et al. 2009). Total injury rates in Barnegat Bay ranged from 18 to 22%, which were similar to the high rate (19.7%) found in the Chesapeake (Roosenburg 1991). Other areas in Florida, North Carolina, and South Carolina, have lower injury rates from 6 to 16% (Hart and McIvor 2008, Cecala et al. 2009). Frequency of injury increased temporally from 2006 to 2011 in Forsythe (Fig. 2-3). This temporal increase may be due to a corresponding increased number of recreational motor boats in Barnegat Bay (MTA 2008).

Diamondback terrapin limb loss rate in Forsythe Refuge (5%), on Sedge Island (1%), and in Spizzle Creek (2%) was lower than that found in other parts of the terrapin

range. In Kiawah Island, 8% of captured terrapins are missing one limb and an additional 0.9% are missing more than one limb (Cecala et al. 2009).

Large diamondback terrapins were more likely to have an anthropogenic injury than small terrapins in Forsythe (Fig. 2-4). Many of the anthropogenic injuries in Forsythe were likely due to recreational boat strikes because nesting beaches are not located near roads in Forsythe. Larger male and female terrapins in IBSP were also more likely than smaller terrapins to have been hit by a boat or an automobile. In IBSP, nesting beaches are located near roads so anthropogenic injuries were likely due to automobiles or recreational boat strikes. Terrapins did not experience reduced mass as a result of anthropogenic injuries in Barnegat Bay (Table 2-2). Cecala et al. (2009) also found no significant decrease in terrapin mass due to major shell damage in Kiawah Island, South Carolina.

Female terrapins were more prone to injuries than male terrapins in all locations. In IBSP, female terrapins are subjected to more sources of injuries because nesting females have to cross roads to get to nesting sites and risk being hit by an automobile. In Forsythe, female terrapins are known to spend more time in the open bay than male terrapins (Sheridan 2010). In the open bay, female terrapins are likely exposed to not only higher numbers of boats, but also longer vessels with larger engines.

Survivorship is the proportion of individuals that reach the next year of life. In Barnegat Bay, survivorship of uninjured male and uninjured female terrapins was not significantly different (Fig. 2-5). In Forsythe, injured male terrapins had significantly lower survivorship than uninjured males, but this was not the case for females. Female terrapins with anthropogenic injuries in Sedge and Spizzle had lower survivorship than uninjured terrapins. Our survivorship estimates were similar to estimates for terrapins on

Kiawah Island, South Carolina (Cecala et al. 2009). Mean uninjured terrapin survivorship in Barnegat Bay was 0.66 for females and 0.72 for males. On Kiawah Island, both males and females had survivorship of approximately 0.8. Injured males in Barnegat Bay (0.1) had much lower survivorship than those in Kiawah Island (0.5).

Anthropogenic injury rates reported here likely underestimate the actual anthropogenic injury rate for terrapins in Barnegat Bay because injured terrapins must have survived injury to be captured. We did not measure mortality rate associated with automobiles or recreational motor boats. Some sea turtle carcasses sink to the bottom of the water column following boat strikes and are never found: this also likely happens to diamondback terrapins (Crowder et al. 1995).

Terrapin populations are already declining in many parts of their range due to anthropogenic impacts such as drowning in crab pots, automobiles hitting nesting females, and predation by non-native predators (Roosenburg et al. 1997, Wood and Herlands 1997, Draud et al. 2004, Szerlag and McRobert 2006, Hart and McIvor 2008). The increasing threat of boat strike injuries will cause even greater declines to terrapin populations. Reducing the boat injury rate of terrapins is essential to maintaining viable terrapin populations. Boaters must be educated about preventative measures to decrease boat strikes on aquatic animals and regulations concerning boat use need to be implemented and enforced.

Table 2-1. Frequency of injured diamondback terrapins. We compared yearly diamondback terrapin injury rates to see if there were temporal trends in Forsythe Refuge, Spizzle Creek, and Sedge Island (ANOVA). Injury rates were divided into three categories: all injury, anthropogenic injury, and missing limb. We found that all injuries and anthropogenic injuries of terrapins increased significantly (*) in Forsythe Refuge from 2006 to 2011.

Location	Type of Injury	Equation	R ²	P-value
Forsythe	All	$Y = 0.02 X - 48.0$	0.68	* 0.04
	Anthropogenic	$Y = 0.01 X - 22.8$	0.82	* 0.01
	Missing Limb	$Y = 0.009 X - 17.2$	0.32	0.24
Sedge	All	$Y = 0.009 X - 18.2$	0.03	0.75
	Anthropogenic	$Y = 0.03 X - 60.1$	0.52	0.11
	Missing Limb	$Y = 0.003 X - 5.2$	0.17	0.42
Spizzle	All	$Y = 0.02 X - 35.7$	0.22	0.29
	Anthropogenic	$Y = 0.006 X - 12.8$	0.02	0.75
	Missing Limb	$Y = 0.005 X - 10.0$	0.23	0.28

Table 2-2. Plastron length and mass of terrapins. Terrapins did not experience significantly reduced mass as a result of anthropogenic injuries (linear regression, ANCOVA).

Location	Sex	Injured	Uninjured	F-value	df	P-value
Forsythe	Males	$Y = 0.01 X + 1.37, R^2 = 0.81$	$Y = 0.01 X + 1.41, R^2 = 0.81$	0.33	700	0.56
Forsythe	Females	$Y = 0.009 X + 1.52, R^2 = 0.98$	$Y = 0.009 X + 1.56, R^2 = 0.97$	2.69	1255	0.10
Sedge	Females	$Y = 0.003 X + 1.46, R^2 = 0.65$	$Y = 0.003 X + 1.46, R^2 = 0.54$	0.02	244	0.88
Spizzle	Females	$Y = 0.008 X + 1.63, R^2 = 0.87$	$Y = 0.009 X + 1.57, R^2 = 0.96$	0.21	374	0.65

Table 2-3. Mean body condition index. Anthropogenic injured terrapins did not have significantly different body condition indices (CI) than uninjured terrapins (Student's t-test, $p > 0.05$). CI values are ± 1 standard deviation.

Location	Sex	CI (Uninjured)	CI (Injured)	P-value
Forsythe	Female	0.54 ± 0.07	0.55 ± 0.06	0.18
Forsythe	Male	0.54 ± 0.04	0.54 ± 0.03	0.34
Spizzle	Female	0.53 ± 0.05	0.51 ± 0.03	0.19
Spizzle	Male	0.54 ± 0.03	0.53 ± 0.04	0.38
Sedge	Female	0.51 ± 0.05	0.54 ± 0.04	0.13

Table 2-4. Cormack-Jolly-Seber (CJS) model results in program MARK. The model took into account yearly survival (Φ) and yearly recaptures (p) probabilities. Differences between injured and uninjured terrapins (g) were taken into account in some models as was temporal variation (t) in survival and recapture probability. QAIC_c was used to determine which model was the best fit and the model with the highest support was $\Phi^{gt}p_t$ for all terrapins in Forsythe Refuge, on Sedge Island, and in Spizzle Creek.

Location	Sex	Model	Number of Parameters	QAIC	QAIC _c
Forsythe	Female	$\Phi^{gt}p_t$	16	901.35	0.997
		Φp_t	19	914.64	0.019
		$\Phi^t p_t$	7	917.93	0.000
Forsythe	Male	$\Phi^{gt}p_t$	8	1552.52	0.875
		Φp_t	6	1557.47	0.001
		$\Phi^t p_t$	9	1564.63	0.000
Sedge	Female	$\Phi^{gt}p_t$	9	575.34	0.860
		$\Phi^t p_t$	7	580.03	0.082
		Φp_t	6	580.78	0.060
Spizzle	Female	$\Phi^{gt}p_t$	2	171.06	0.780
		Φp_t	4	174.41	0.150
		$\Phi^t p_t$	4	175.83	0.071



Fig. 2-1. Study site. Terrapins were captured in Edwin B. Forsythe Wildlife Refuge (Forsythe) and Island Beach State Park (IBSP) in Barnegat Bay, New Jersey, USA. The two study sites in IBSP were Sedge Island and Spizzle Creek.

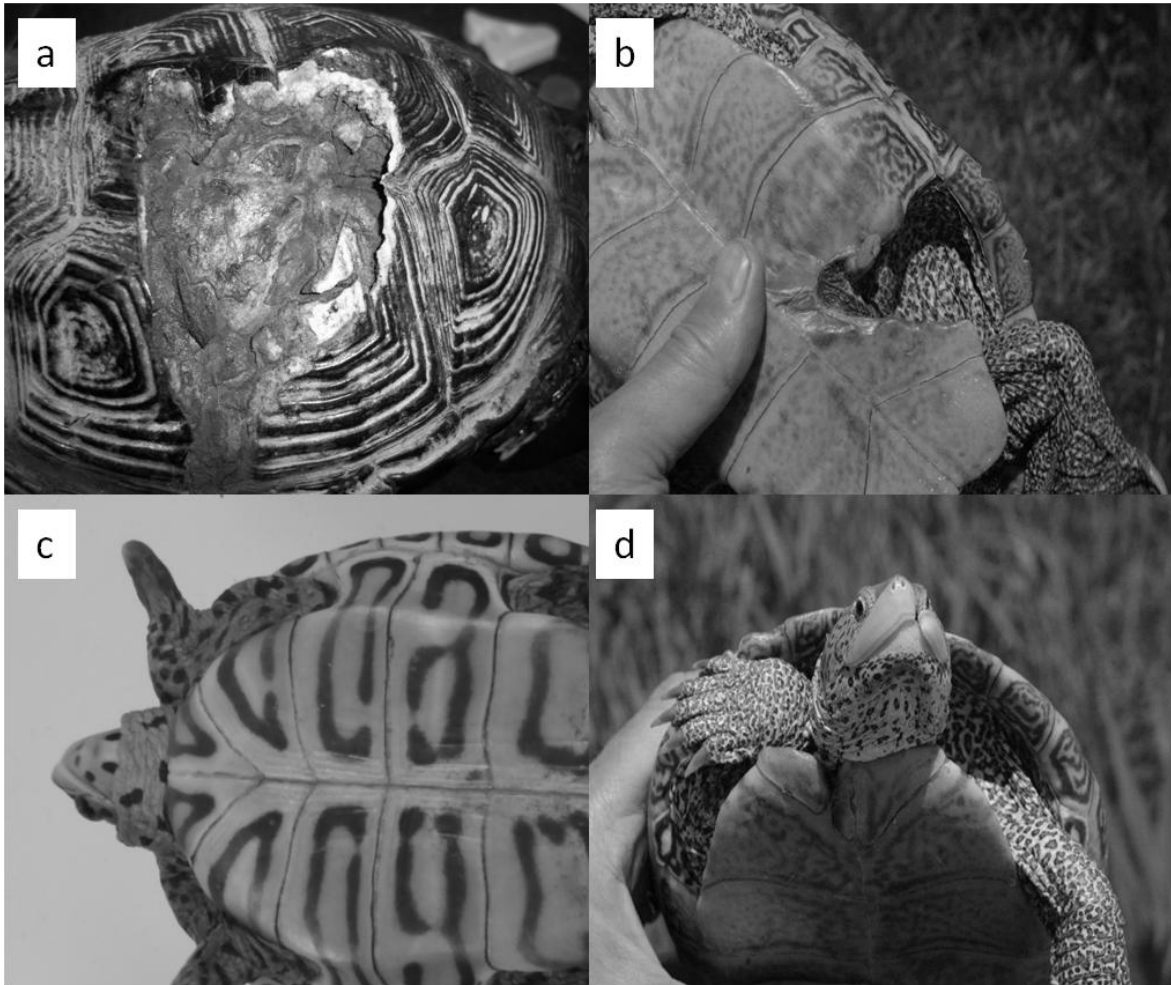


Fig. 2-2. Anthropogenic injuries to diamondback terrapins. Diamondback terrapins were classified as having an anthropogenic injury if damage occurred to two or more vertebral or costal scutes (a), two or more plastral scutes (b), and/or three or more marginal scutes. Many injured terrapins also had missing limbs (c), tail, or head (d) injuries. Anthropogenic injury rates are likely an underestimate of the actual number of terrapins hit by boats and automobiles because many of these injuries lead to mortality.

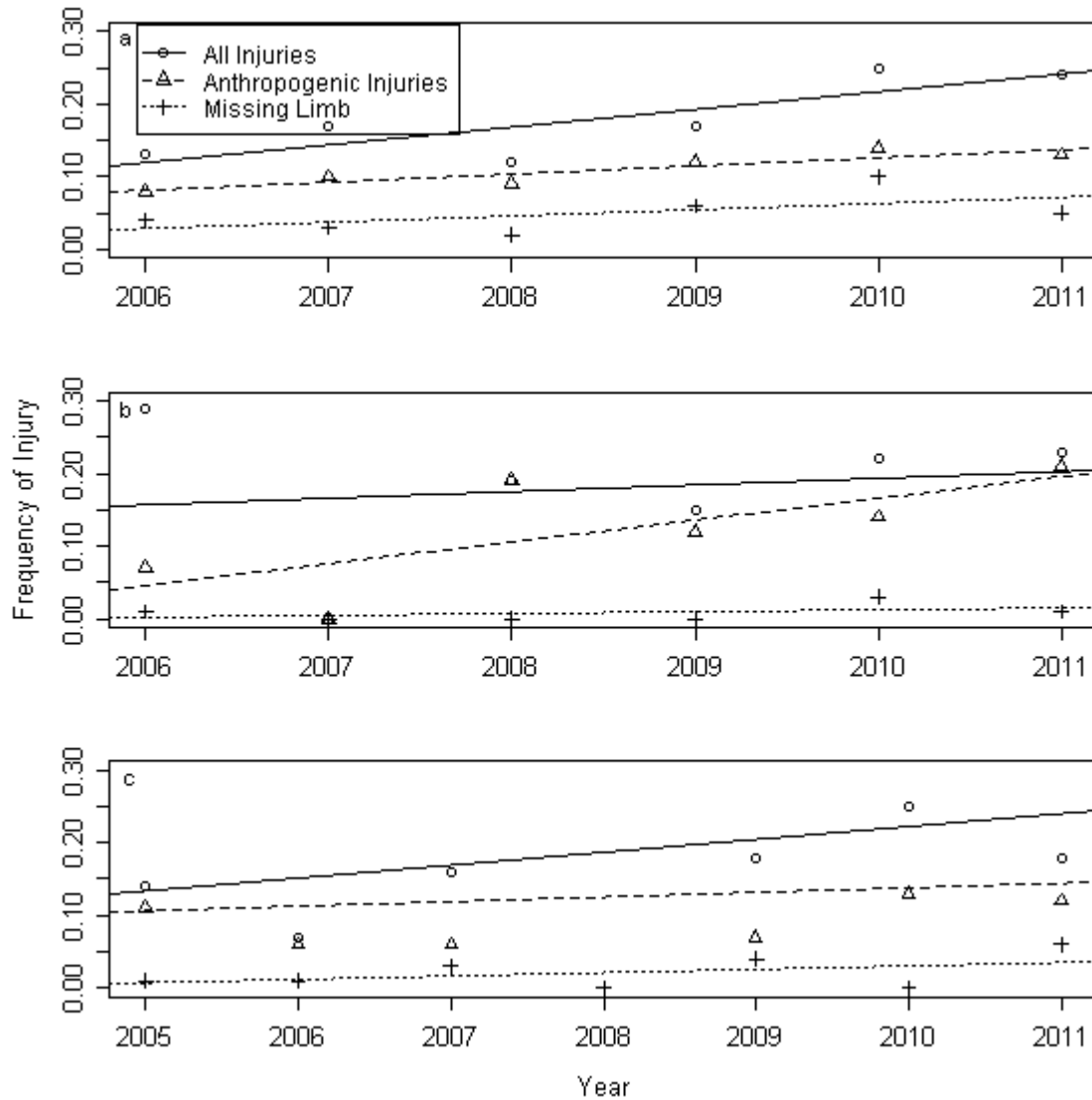


Fig. 2-3. Frequency of injured terrapins. Diamondback terrapins have sustained substantial injuries, anthropogenic injuries, and missing limbs in Forsythe Refuge (a), Sedge Island (b), and Spizzle Creek (c). All injury ($Y = 0.02 X - 48.02$, $R^2 = 0.68$, $P = 0.04$) and anthropogenic injury ($Y = 0.01 X - 22.8$, $R^2 = 0.82$, $P = 0.01$) rates increased temporally in Forsythe from 2006 to 2011.

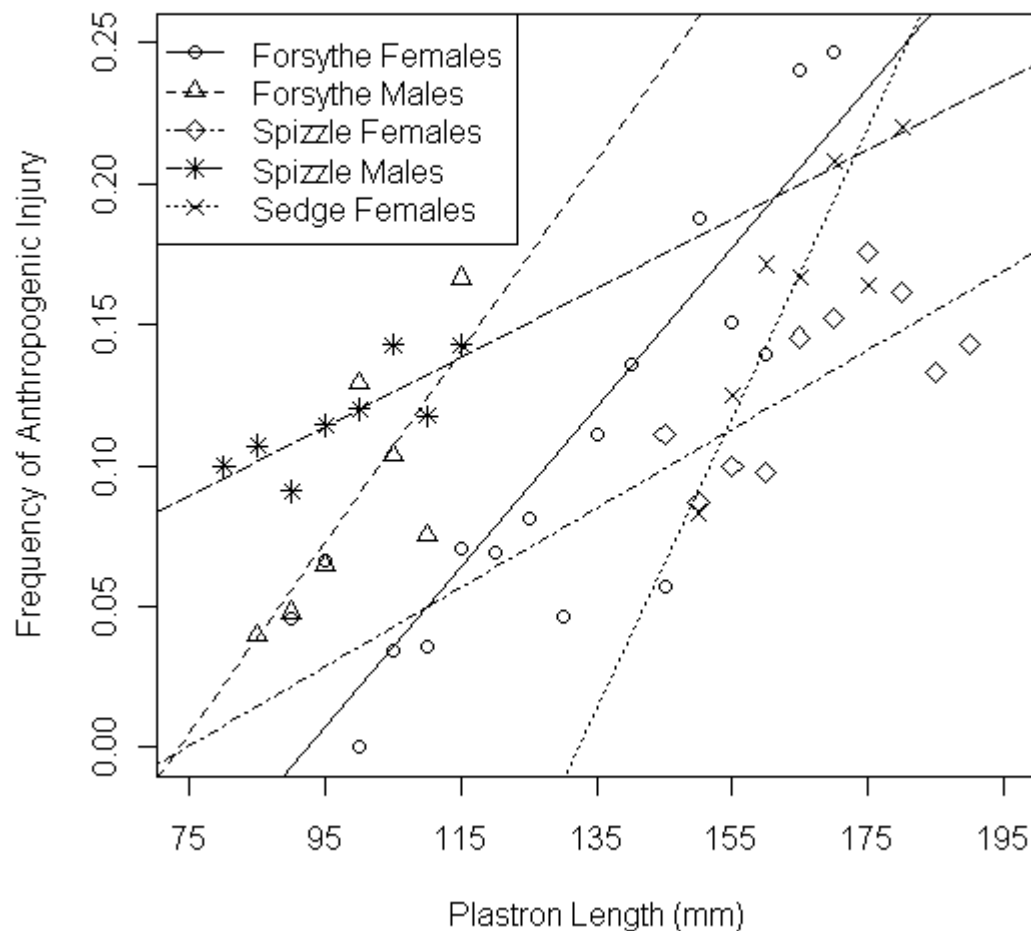


Fig. 2-4. Frequency of anthropogenic injury and plastron length. Frequency of anthropogenic injury increased significantly with plastron length for female ($P = 4.0 \times 10^{-6}$) and male ($P = 0.03$) terrapins in Forsythe. Frequency of anthropogenic injury also increased in Sedge ($P = 2.9 \times 10^{-4}$) and Spizzle ($P = 0.02$ females; $P = 0.02$ males). Plastron length from the individual's first capture was used. Frequencies of injuries are the mean value of 5 mm intervals.

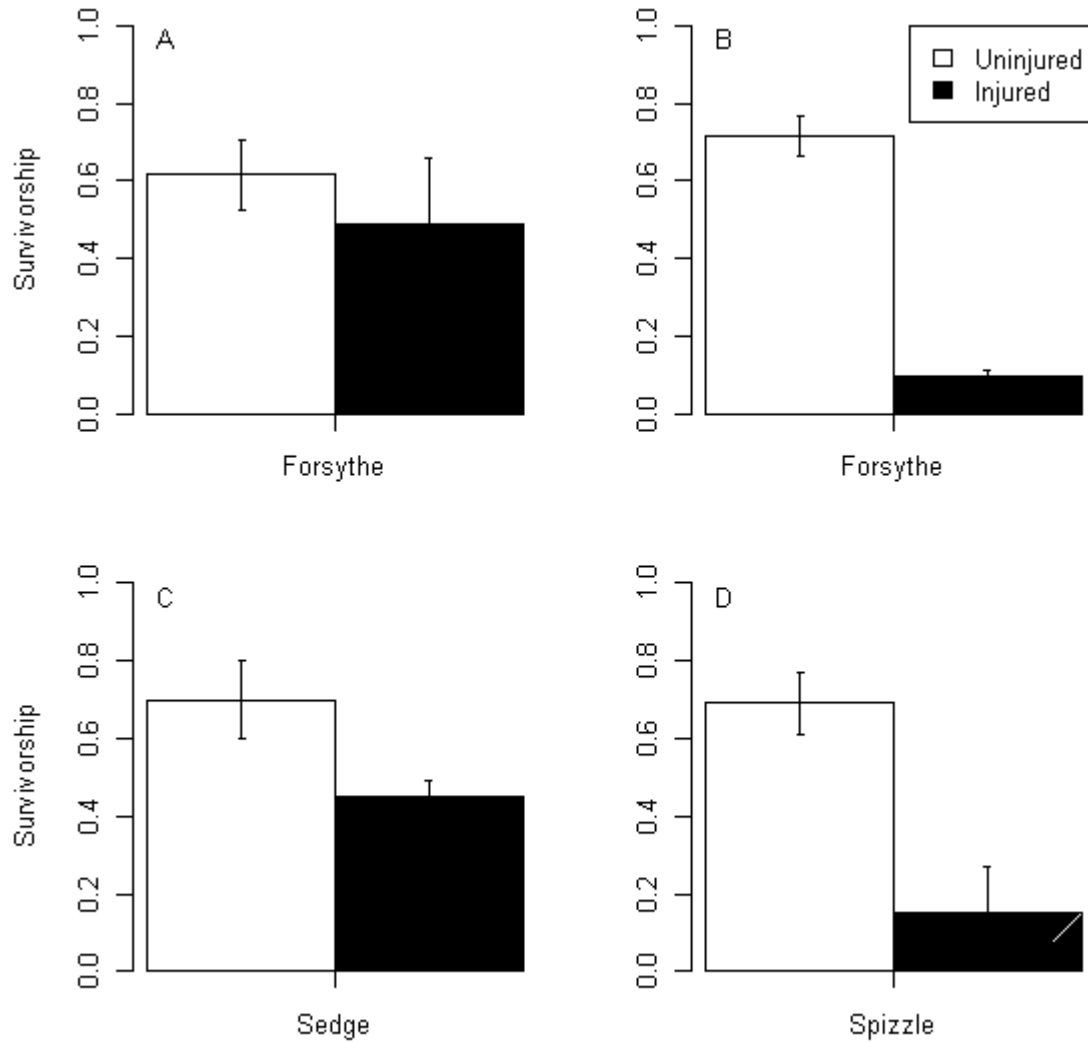


Fig. 2-5. Survivorship of anthropogenic injured and uninjured terrapins. We estimated survivorship with a Cormack-Jolly-Seber (CJS) model using program MARK. Estimates were from the best-fit model, $\Phi^{gt}p_t$, for both female and male terrapins in all locations. In Forsythe, female terrapins (A; $P = 0.32$) with anthropogenic injuries did not have lower survivorship than uninjured terrapins. Male terrapins (B; $P = 0.02$) with anthropogenic injuries in Forsythe did have significantly lower survivorship than uninjured males. Anthropogenic injured female terrapins in Sedge (C; $P = 0.04$) and Spizzle (D; $P = 0.008$) had lower survivorship than uninjured terrapins. Error bars are ± 1 standard error.

CHAPTER 3: AERIAL SOUND DETECTION BY DIAMONDBACK TERRAPINS USING AUDITORY EVOKED POTENTIALS

Abstract

Sound detection is essential for many organisms because hearing allows animals to perform important tasks such as communicating, avoiding predators, finding mates, navigating, and finding food. As anthropogenic sounds increase in intensity in many habitats, a better understanding of how animals hear and process acoustic stimuli is needed. In this study, we used auditory evoked potentials to study sound detection by diamondback terrapins (*Malaclemys terrapin*). Computed tomography (CT) scans allowed us to calculate the volume of air in the middle ear in order to estimate its resonant frequency. Terrapins responded to low-frequency sounds (100 to 1000 Hz) in air, with the range of best hearing from 400 to 600 Hz, and with a minimum threshold of 64 dB re 20 μ Pa Sound Pressure Level (SPL). Calculated resonance frequencies were approximately 900 Hz for the smaller male terrapin and 600 Hz for the larger female. Using in air audiograms, CT scans, and previous research on the red-eared slider turtle, we estimated underwater hearing capability of terrapins and found that frequencies of best hearing were 200 to 500 Hz with lowest threshold of 70 dB re 1 μ Pa SPL. Many aerial and underwater anthropogenic sound sources produce acoustic signals that overlap with the hearing capability of terrapins. Future research should focus on whether they behaviorally or physiologically respond to anthropogenic sounds within their hearing range.

Introduction

The sense of hearing is essential for many animals because sounds are used for purposes including communication, finding mates, avoiding predators, finding food, and

navigating (Wever 1978). Originally, researchers assumed that turtles had poor hearing because they lack an external ear and rarely vocalize (Wever 1978, Lenhardt 1981). In place of an external ear, turtles have a tympanic membrane which cannot be visually distinguished from the skin on the side of the head. Behavioral and physiological research, however, suggest that various freshwater and marine turtle species respond to low-frequency sounds (Bartol et al. 1999, Christensen-Dalsgaard et al. 2012).

Hearing has been studied physiologically in freshwater and marine turtles by recording auditory evoked potentials (AEPs) in red-eared slider (*Trachemys scripta elegans*), loggerhead (*Caretta caretta*), green (*Chelonia mydas*), and Kemp's ridley (*Lepidochelys kempii*) turtles (Bartol et al. 1999, Bartol and Ketten 2006, Christensen-Dalsgaard et al. 2012). Red-eared slider turtles respond to low-frequency sounds in air and underwater (Christensen-Dalsgaard et al. 2012). In air, the range of best hearing for red-eared slider turtles is 300 to 500 Hz with lowest threshold of 60 dB re 20 μ Pa SPL. The range of best hearing underwater for red-eared slider turtles is 400 to 500 Hz with lowest threshold of 80 dB re 1 μ Pa SPL. Loggerhead sea turtles respond to aerial sounds under the frequency of 1000 Hz (Bartol et al. 1999). Juvenile Kemp's ridley turtles respond to underwater sounds from 100 to 500 Hz (Bartol and Ketten 2006). Sub-adult green turtles respond to 100 to 500 Hz sounds underwater with best sensitivity from 200 to 400 Hz and juvenile green turtles respond to 100 to 800 Hz with best sensitivity from 600 to 700 Hz.

Many turtles have an air-filled middle ear (Lenhardt 2002). The air in the middle ear has a specific resonance that allows the system to oscillate at greater amplitudes at certain frequencies (Rossing et al. 2002, Christensen-Dalsgaard et al. 2012). By measuring the volume of air in the middle ear, resonance frequency of the middle ear can be

calculated (Wever and Vernon 1956). The resonance frequency of the middle ear in red-eared slider turtles (*Trachemys scripta elegans*) is approximately 500 Hz (Christensen-Dalsgaard et al. 2012).

No studies exist that focus on the hearing capability of brackish water species such as diamondback terrapins (*Malaclemys terrapin*). Diamondback terrapins are habitat generalists that utilize both aquatic and terrestrial habitats for activities such as foraging, mating, basking, and nesting so hearing is likely useful in air and underwater. Terrapins live in estuaries that have high levels of anthropogenic sounds created by recreational and commercial boats. Anthropogenic sounds can affect aquatic animals in many ways by causing behavioral changes, masking other natural sounds, and leading to hearing loss, tissue damage, or stranding (Southall et al. 2000, Popper 2009). Physiological responses to anthropogenic sounds include increased heart rate and stress levels (Bowles et al. 1999). Limited research has been conducted on the behavioral and physiological effects of anthropogenic sounds on turtles. Prior to understanding the effects of anthropogenic sounds on marine animals, the hearing sensitivity of the animals must be assessed.

In order to understand the effects of anthropogenic sounds on terrapins, a basic understanding of the hearing capability of terrapins is essential. We conducted this study to determine the hearing capability of diamondback terrapins in air by recording AEPs. Based on past research on marine and freshwater turtles, we hypothesized that terrapins would be able to hear a range of sounds below 1000 Hz. In addition, we calculated the air volume of the terrapin middle ear to determine resonance frequency (Christensen-Dalsgaard et al. 2012). Underwater hearing capability of terrapins was also estimated based on CT scans and in air hearing audiograms. Some anthropogenic sounds, such as those created by boat

recreational and commercial boat engines, have a typical dominant frequency range of 1 to 2000 Hz (Richardson et al. 1995). If terrapins hear low frequency sounds, then their hearing range overlaps with these anthropogenic sounds, which may affect their survival.

Materials and Methods

Auditory Evoked Potentials

We recorded AEPs in air for five diamondback terrapins including two adult males (296 g and 320 g), two adult females (1200 g and 1410 g), and one juvenile (200 g). The terrapins were provided by the Marine Academy of Technology and Environmental Science (MATES) in Manahawkin, New Jersey, USA. When the animals were obtained, they underwent a health evaluation by a New Jersey State Rehabilitation specialist and were found to be in excellent health.

We lightly anesthetized terrapins prior to trials with a combination of ketamine (30 mg/kg in males and juveniles, 35 mg/kg in females) and xylazine (20 mg/kg) injected into rear limb muscle. Diamondback terrapins were placed on a sponge on a table to decrease vibrations. We quantified acoustic stimuli by recording AEPs and our methods were similar to those used in previous studies (Higgs et al. 2002, Brittan-Powell et al. 2005, Christensen-Dalsgaard et al. 2012). We inserted three stainless steel electrodes (impedance approximately 1 k Ω) subdermally in the terrapin: recording (above the brainstem), reference (placed above tympanum), and ground (placed in right forelimb muscle). We recorded electrode signal through a Tucker-Davis Technologies (TDT) low-impedance headstage and preamplifier (PA4, RA4), and processed with a digital signal processor (RM2). Custom-made software (QuickABR) previously used to measure AEPs in red-eared slider turtles (*Trachemys scripta elegans*) and longfin squids (*Loligo pealeii*) was

used for stimulation, recording, and data analysis (Brandt et al. 2008, Mooney et al. 2010, Christensen-Dalsgaard et al. 2012).

We stimulated terrapins closed-field, i.e., a coupler was sealed over the tympanum with ear mold compound (Gold Velvet II; All American Mold Laboratories, Oklahoma City, OK). The coupler consisted of a headphone for sound presentation (Beyer 48.0A) and a microphone (Brüel & Kjaer 1/2”) for calibration. Ordinarily, the problem with measuring AEPs is that click stimuli are broadband sounds thus the measurement is not frequency specific. To minimize this problem, we used a masking method by measuring AEPs using a broadband click and then measuring AEPs with the same click and a narrow band masker (Brandt et al. 2008). The difference between these two AEPs was a measure of sensitivity to the narrow-band masker. This technique allowed us to measure auditory response at low frequencies which is difficult with tone burst AEP. Diamondback terrapins were exposed to various frequencies (100, 200, 300, 400, 500, 600, 700, 800, and 1000 Hz) and sound pressure levels (SPLs). Hearing thresholds were determined using the visual detection technique. We looked at the difference signal and determined threshold as the lowest SPL for each frequency where the signal could still be seen in the time domain and Fast Fourier Transform (FFT), and not hidden in background noise.

CT Scans

We used Computed Tomography (CT) head scans to record ear morphology of two diamondback terrapins (male and female) at the Woods Hole Oceanographic Institution CT imaging facility with the help of Dr. Darlene Ketten and Ms. Julie Arruda. These terrapins were full grown adults and were representative of the mean size of terrapins captured in the wild. Diamondback terrapins are a sexually dimorphic species with males being

substantially smaller than females. The male terrapin had a mass of 320 g and a straight carapace length (SCL) of 121 mm, whereas the female had a mass of 1200 g and a SCL of 155 mm. Terrapins were anesthetized as above and placed in a box under a damp towel. The CT scans were obtained in a Siemens Volume Zoom CT scanner.

We used the software program ImageJ to estimate middle ear volume for each terrapin by using the polygon analysis tool to estimate middle ear area for each CT scan slice. This area was then multiplied by the thickness of each slice (0.5 mm) and the areas added together to estimate the total volume of each middle ear. We calculated resonance frequencies using a generalized formula for resonance frequency of a spherical air bubble (Urlick 1983, Christensen-Dalsgaard and Elepfandt 1995):

$$f_{res} = \frac{0.327}{\sqrt[3]{\frac{3 \cdot v}{4 \cdot \pi}}}$$

where f_{res} is resonance frequency of the bubble and v is volume of cavity (cm^3). The middle ear cavity of diamondback terrapins, and many other turtle species, is a curved ellipsoid that scales with head size (Willis et al. 2011). Since turtles respond to mostly sounds under 1000 Hz, cavity volume was assumed to matter more than cavity shape in determining best resonance frequency.

Underwater Hearing Sensitivity

Under the assumption that terrapin underwater hearing (like hearing in the red-eared slider) is dominated by properties of enclosed air in the middle ear cavity, we estimated underwater sound sensitivity from CT scans and in air audiograms. We assumed that the middle-ear transfer function in air was almost flat (following Ruggero and

Temchin 2002), so the air audiogram reflected the response of the inner ear. A model predicting air bubble vibrations in water has been developed for fish swimbladders (Alexander 1966), although others have argued that surrounding structures such as the middle ear walls may dampen the response which would result in a less sharply tuned response than an air bubble (Tavolga 1964). Following Christensen-Dalsgaard et al (2012), we assumed that these vibrations were driving the eardrum.

Results

Auditory Evoked Potentials

The AEP waveforms of diamondback terrapins had two to three prominent peaks that occurred within the first 10 ms following onset of stimulus (Fig. 3-1). Peak amplitude increased and peak latency decreased as stimulus SPL increased. Mean terrapin AEP audiogram in response to closed-field stimulation was U-shaped with sensitivity from 100 to 1000 Hz (Fig. 3-2). The best sensitivity to sound in air was from 400 to 600 Hz, with lowest threshold of 64 dB re 20 μ Pa SPL. Terrapin sensitivity to the acoustic stimulus decreased sharply after 700 Hz. At the lowest and highest frequencies (100 and 1000 Hz), threshold increased to approximately 85 dB re 20 μ Pa SPL.

CT Scans

The CT scans revealed a cartilaginous tympanic disk under the tympanic membrane (Fig. 3-3). The middle ear bone (extracolumella) was embedded in the tympanic disk and extended through the middle ear to the inner ear. The volume of the male terrapin's middle ear was 0.19 mL and the volume of the female's middle ear was 0.59 mL. The resonance frequency of air bubbles with this volume would be approximately 900 Hz for the male and 600 Hz for the female.

Underwater Hearing Sensitivity

Underwater audiograms were estimated for the same two terrapins used for CT scans (Fig. 3-4). Best sensitivity to underwater sound for female terrapin was from 300 to 500 Hz with lowest threshold at 78 dB re 1 μ Pa. Best sensitivity to underwater sound for the male terrapin was from 200 to 400 Hz with a minimum threshold at 70 dB re 1 μ Pa.

Discussion

The AEP allowed us to evaluate hearing in diamondback terrapins in air. The AEP audiograms accurately predicted shape of behavioral audiograms but they did not predict absolute auditory sensitivity. Thresholds obtained via AEP are typically 10 to 30 dB greater than those obtained through behavioral methods (Gorga et al. 1988, Brittan-Powell et al. 2002). This difference exists because the synchronous nerve activity necessary to elicit an AEP response is higher than that necessary for a behavioral response. Thus diamondback terrapins would likely respond behaviorally to lower sound thresholds than reported here. Other freshwater (Corwin et al. 1982, Christensen-Dalsgaard et al. 2012) and marine turtle species (Ridgway et al. 1969, Lenhardt et al. 1983, Bartol et al. 1999) respond to a narrow range of low-frequency sounds in a similar range to terrapins.

Diamondback terrapins respond physiologically to low-frequency sounds in air. Hearing allows an animal to pick out important acoustic cues from the various simultaneous acoustic cues present in the environment (Bregman 1990, Fay and Popper 2000). Aerially transmitted anthropogenic sounds may mask essential natural sounds reducing an animal's ability to hear the biologically relevant sounds such as an approaching predator (Erbe 2002). Furthermore, animals that are exposed to anthropogenic

high-intensity sounds may suffer from permanent or temporary threshold shift which decreases their hearing capability (Brattstrom and Bondello 1983, Erbe 2002).

The female terrapin had a larger middle ear cavity than the male terrapin (Fig. 3-3). Since terrapins are a sexually dimorphic species, we expected this difference in ear cavity size (Willis et al. 2011). Red-eared slider turtles have similarly sized middle ear cavities (0.22 mL in small turtle, 0.50 mL in large turtle) to terrapins (Christensen-Dalsgaard et al. 2012). The clawed frog ear closely resembles the turtle ear with a cartilaginous disk and air-filled middle ear that is adapted for underwater hearing (Christensen-Dalsgaard and Elepfandt 1995). Diamondback terrapins have larger middle ear cavities than clawed frogs (*Xenopus laevis*, 0.013 to 0.034 mL) which we expected since clawed frogs are smaller than terrapins. The clawed frog ear's peak frequency (~2.4 kHz) is also higher than the terrapin ear's peak frequency.

Diamondback terrapins are habitat generalists and spend substantial time in the water and on land. Acoustic stimuli are transmitted with different physical characteristics through various types of media (Kalmijn 1988). Underwater, sound waves transmit pressure and displacement information to the ear. In air, acoustical information is transmitted solely as pressure waves unless it is very close to the source. Thus, it is not appropriate to assume that an organism will have the same hearing capacity underwater as it does in air. Although hearing thresholds measured in air do not equate to absolute underwater hearing thresholds, we were able to estimate the underwater hearing capability of terrapins (Fig. 3-4). Future studies should record AEPs in terrapins underwater.

Underwater anthropogenic sound sources, such as recreational boats, commercial vessels, and dredging barges, are of concern to diamondback terrapins because these

sounds overlap with the estimated underwater hearing range. Estuarine organisms may behaviorally or physiologically respond to anthropogenic sound sources in detrimental ways such as avoiding breeding or feeding areas which could negatively affect their life history traits and lead to declines in survival. Further research is underway regarding the effects of anthropogenic sounds on diamondback terrapins and other organisms in estuarine habitats.

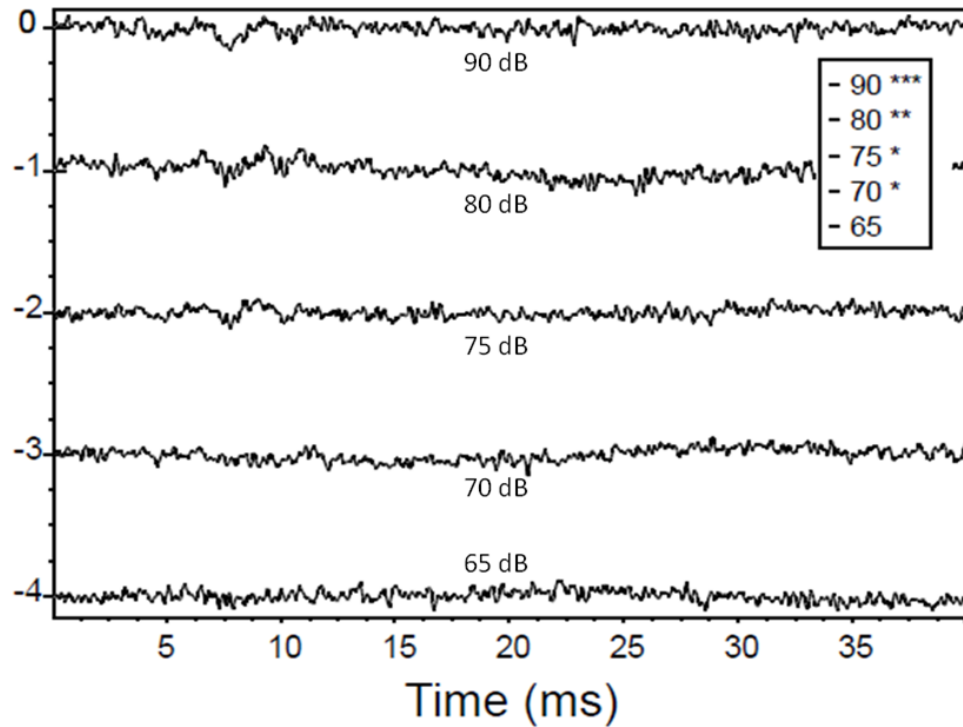


Fig. 3-1. Representative AEP waveform. Diamondback terrapin hearing sensitivity in air was measured through auditory evoked potentials (AEP). In response to a 400 Hz tone, the juvenile terrapin was exposed to five different masker amplitudes (calculated as dB re 20 μ Pa SPL). The AEP difference signal decreased from top to bottom. Hearing thresholds were calculated from curves such as these when the response of the terrapin was greater than the average noise floor.

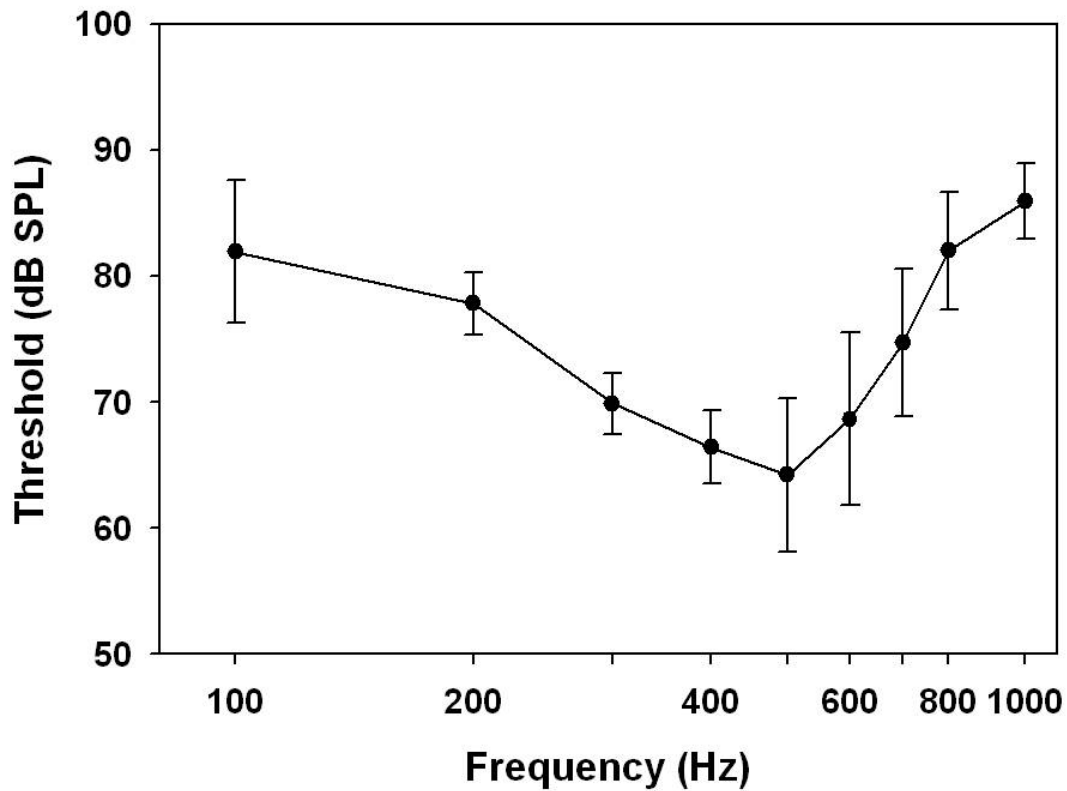


Fig. 3-2. Audiogram for airborne sound. Auditory evoked potentials (AEP) were measured from terrapins ($n = 5$) in air that were stimulated with a pulse and a pulse plus tonal masker. Masker sensitivity was determined by subtracting masked from unmasked responses. The curve is the measurement of mean thresholds in response to closed-coupler stimulation. Thresholds plotted are sound pressure levels (SPL) in dB re 20 μ Pa. Error bars are ± 1 SD.

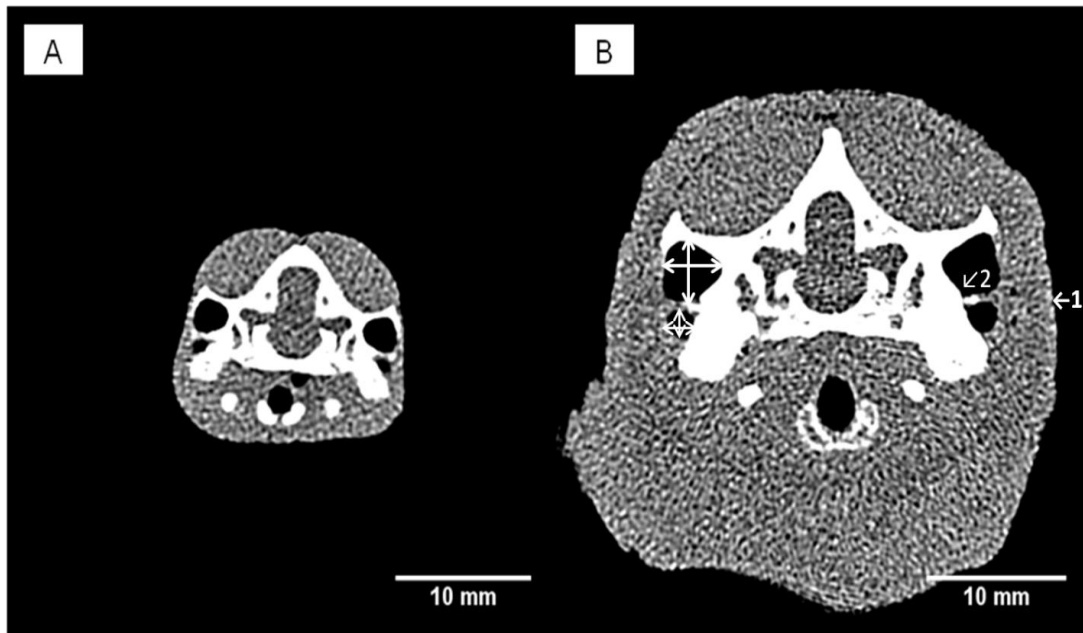


Fig. 3-3. CT scan of diamondback terrapin skull. CT scans were completed on two terrapins (A - male; B - female). The cartilaginous tympanic disk (1) was under the tympanic membrane. The middle ear bone (2) was imbedded in the tympanic disk. Area of the middle ear (depicted with arrows) was measured in ImageJ and multiplied by slice thickness (0.5 mm) to calculate volume. Slice volumes were summed to calculate total middle ear cavity volume. The volume of the middle ear cavities were 0.19 mL in the male and 0.59 mL in the female. Numbers are representative of same morphological structure in both (A) and (B).

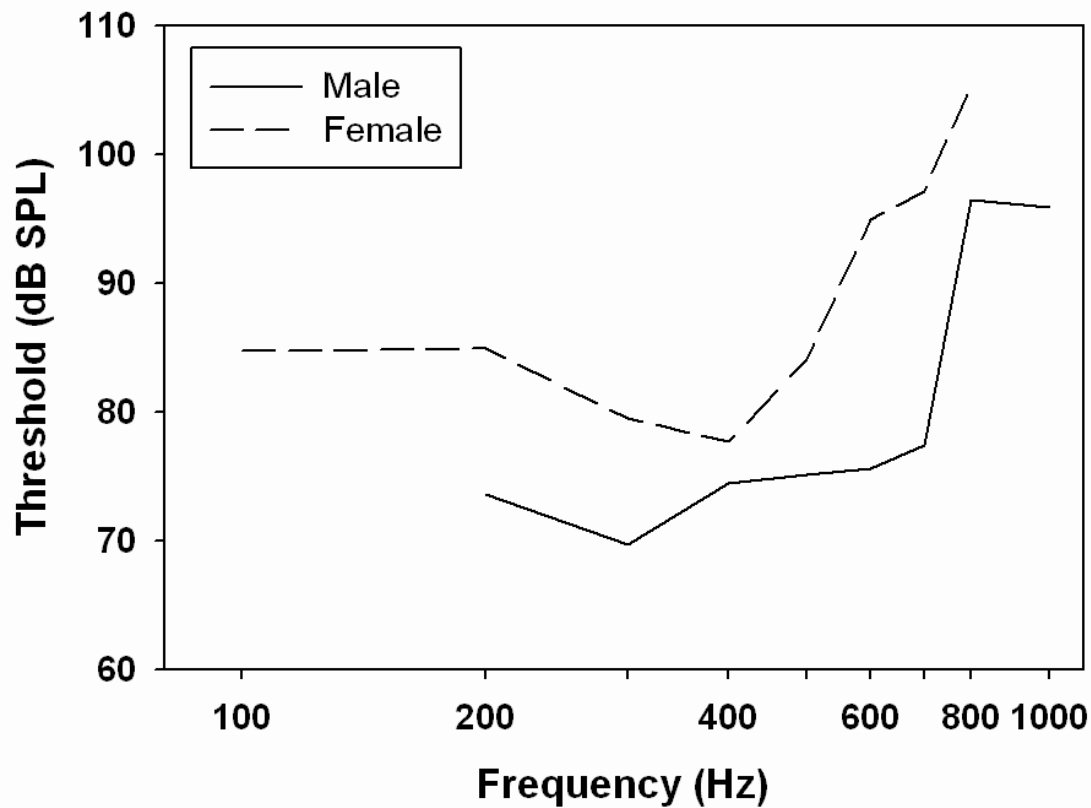


Fig. 3-4. Underwater hearing estimate. Underwater hearing capability of two terrapins (same individuals depicted in Fig. 3-3) was estimated with the assumption that underwater hearing was dominated by properties of enclosed air in the middle ear. Thresholds plotted are sound pressure levels (SPL) in dB re 1 μ Pa.

CHAPTER 4: AMPHIBIOUS HEARING CAPABILITY OF DIAMONDBACK TERRAPINS STUDIED WITH AUDITORY EVOKED POTENTIALS

Abstract

Anthropogenic sounds are increasing in many estuarine environments due to recreational boats. As anthropogenic sounds increase in many habitats, a clearer understanding of how animals are affected by human-generated sounds is necessary. We determined hearing range of the diamondback terrapin (*Malaclemys terrapin*) and determined whether it overlaps with anthropogenic sounds produced by recreational motor boats. We recorded auditory evoked potential (AEP) responses in terrapins in air (n = 8) and underwater (n = 5) to determine hearing capability. We also measured sound pressure levels (SPLs) from recreational boats and personal watercraft (PWC) in Barnegat Bay, New Jersey, USA. Terrapins responded to sounds in air from 50 to 1600 Hz with mean lowest threshold of 50 dB SPL re 20 μ Pa. Female terrapins responded to underwater sounds from 50 to 800 Hz with mean lowest threshold of 86 dB SPL re 1 μ Pa. Recreational boat and PWC sound recordings contained low-frequency sounds at SPLs that are within the hearing range of terrapins. Future research should focus on whether terrapins and other estuarine organisms behaviorally and physiologically respond to anthropogenic sounds.

Introduction

Hearing is essential for many species because it allows an organism to be aware of its surroundings. Hearing acoustic stimuli is necessary for essential activities such as locating mates/food and avoiding predators. Initially, there was doubt that turtles could hear because they lack external ears (Wever 1978, Lenhardt 1981). Physiological and behavioral studies have since shown that many marine and freshwater turtle species can

hear low-frequency sounds less than 1000 Hz (Bartol et al. 1999, Christensen-Dalsgaard et al. 2012).

Anthropogenic sounds, such as those produced by recreational boats, are increasing in intensity in many aquatic environments. Boat sounds consist of a broad range of frequencies up to 20,000 kHz (Richardson et al. 1995), but when boats travel slowly, dominant sounds are below 1,000 Hz (Richardson and Wursig 1997). Small outboard motorboats have the highest sound pressure level (SPL) when compared with other types of watercrafts (Haviland-Howell et al. 2007). Anthropogenic sounds from small recreational boats may affect animals in various detrimental ways. Prior to studying the effects of anthropogenic sounds on aquatic animals, underwater hearing capability must be analyzed to determine if overlap exists between hearing range and anthropogenic sounds.

Auditory evoked potentials (AEPs) are often measured to determine the hearing capability of reptiles because these techniques are non-invasive and require no behavioral training (Bartol and Ketten 2006). AEPs are measured by recording the evoked neural response to an acoustic stimulus being presented to an organism. Various frequencies and intensities of sounds are presented to the animal until the threshold is reached where the neural response is no longer apparent. AEPs are commonly used to measure sensitivity, receptive range, and intensity range of hearing in turtles (Manley 1971).

Diamondback terrapins (*Malaclemys terrapin*) are habitat generalists spending significant time in terrestrial and aquatic environments and thus need to be able to interpret acoustic stimuli in both environments. Amphibious hearing has been studied in few organisms with the exception of red-eared slider turtles (*Trachemys scripta elegans*) and pinnipeds (Kastak and Schusterman 1998, Christensen-Dalsgaard et al. 2012). The

objective of this study was to determine the hearing capability of terrapins in air and underwater using the AEP technique. We also determined whether the underwater hearing range of terrapins overlaps with the sounds of recreational boats and personal watercrafts (PWC).

Materials and Methods

Diamondback Terrapins

We recorded AEPs of adult diamondback terrapins in air ($n = 8$, two males, six females) and underwater ($n = 5$). Terrapins are a sexually dimorphic species with adult females being significantly larger than adult males. We were only able to measure underwater hearing in female diamondback terrapins because terrapins were anesthetized, intubated, and suspended underwater during trials. The custom-made endotracheal tubes (described below) were too small for male terrapins, thus underwater audiograms were created only for female terrapins.

Anesthesia

We anesthetized terrapins with a combination of ketamine (in air 8-12 $\mu\text{g/kg}$; underwater 12 $\mu\text{g/kg}$) and dexmedetomidine (in air 40-60 $\mu\text{g/kg}$; underwater 60 $\mu\text{g/kg}$). During underwater trials, we intubated diamondback terrapins with custom-made endotracheal tubes (MILA International, Inc., Erlanger, Kentucky, USA) that were cuff inflated with 0.4 mL air and ventilated with a 60 mL catheter-tipped syringe as was previously done in green sea turtles, *Chelonia mydas* (Harms et al. 2009). The endotracheal tubes were made with an inner diameter of 6 mm, a wall thickness of 2 mm, and were 50 cm long. Post an AEP trial, dexmedetomidine was reversed with antipamezole (in air 400-600 $\mu\text{g/kg}$, underwater 600 $\mu\text{g/kg}$).

Auditory Evoked Potentials

We recorded AEPs from diamondback terrapins in air and underwater. The computer program, SigGen (Tucker-Davis Technologies, TDT, Alachua, FL, USA), was used to create the signals. Diamondback terrapins were presented with pulsed tones including the following frequencies: 50, 100, 200, 400, 600, 800, 1000, 1200, and 1600 Hz. Acoustic stimuli were gated with a Hanning window and played with the computer program, BioSig (TDT). The stimulus was played 11 times per second, with a rise and fall time of 15 ms and a total presentation time of 85 ms. Maximum sound pressure level (SPL) of each presented frequency ranged from 79 to 113 dB re 20 μ Pa in air and from 124 to 140 dB re 1 μ Pa underwater. Terrapins were first exposed to SPLs of each frequency that were below threshold (i.e., the level at which the terrapin no longer responded to the stimulus) and then SPL was increased in 6 dB steps until threshold was visually detected in the digital signal (Fig. 4-1). Signal presentations (~1,000) were averaged to measure AEP at each frequency that was presented to each terrapin.

Our setup was similar for aerial and underwater hearing trials. We used a custom built Tucker-Davis Technologies (TDT) system for presenting acoustic stimuli, recording evoked neural response, and calibration. TDT system consisted of an enhanced realtime processor (RP 2.1), a Medusa base station (RA 16), a connection to laptop (UB-4), and a power supply (PS25F). The Medusa base station was connected to a four channel Medusa pre-amp (RA4PA) and a RA4L1 for connection of the electrodes. Three disposable needle electrodes (6 mm) were used for grounding (in rear limb of terrapin or water), referencing (neck), and recording (head). All needle electrodes were inserted subdermally the full 6 mm into the terrapin. Post AEP, we calibrated with a real-time processor (RP2) and a

hydrophone (High Tech, Inc., HTI-96-MIN, -240 dB re 1V/ μ Pa to -165 dB re 1V/ μ Pa, 0.02 to 30 kHz) or a microphone placed where the turtle's ear was during the trial.

Aerial Hearing

During aerial hearing trials, we placed terrapins on foam 40 cm from the center of the speaker. We presented acoustic stimulus via a disappearing in-wall speaker (DI 6.5R, Definitive Technology, Baltimore, MD 21117) which was connected to a Samson amplifier. The speaker was suspended by string from a stand made from PVC pipe on a separate table than the terrapin to decrease potential vibrations. After AEP trials were completed, we calibrated the speaker using a LinearX Microphone (Precision Acoustic Measurement Mic, Mode #M31, Serial #160288, Portland, Oregon 97224). We also used the microphone to make two 10 s background noise recordings with the Matlab program, Field Log (D. Mann, University of South Florida).

Underwater Hearing

For underwater AEP trials, the test tank was a 208 liter drum (56 cm diameter X 91 cm height) that was filled with brackish water (15.5 ppt; 85 cm water depth). The test tank was placed on 9 cm of Pactiv square edge insulation (Lowe's Home Improvement, Morehead City, NC, USA) in order to reduce vibrations. The insulation was cut into 30 cm by 30 cm squares and stacked six high (each 1.5 cm in width) with a total of four stacks of insulation under the tank. The test tank was grounded by running a 10 m copper wire from the water into the soil outside of the laboratory. Diamondback terrapins were suspended on a Lycra sheet with their tympanic membranes 10 cm under the surface of the water. We suspended an underwater speaker (Clark Synthesis Tactile Sound, AQ339-Aquasonic) with

string 2 cm from the bottom of the tank. Calibration and background noise were measured following trials with a hydrophone at the location of the terrapin's ear.

Creating Audiograms

We visually determined thresholds in air and underwater for each diamondback terrapin at various frequencies from 50 to 1600 Hz. AEP thresholds were defined as the lowest SPL where the electrophysiological response (visualized as peaks that occurred at twice the frequency of acoustic stimulus) was still visible in the time domain and fast Fourier transform without being hidden by the background sounds (Fig. 4-1).

Anthropogenic sounds in Barnegat Bay, New Jersey

We recorded underwater sounds produced by a PWC (Yamaha WaveRunner, 3 m, 100 hp, 64 km/hr) and four recreational boats (Lowe Boat, 4.3 m, 9.9 hp, 23.3 km/hr; Polar Kraft, 4.3 m, 25 hp, 41.9 km/hr; Action Craft, 5.5 m, 110 hp, 45.4 km/hr; Parker Boat, 8.5 m, 250 hp, 53.6 km/hr) in Barnegat Bay, New Jersey, USA. We made recordings with a digital recording computer (Sound DSA ST 191; Cetacean Research Technology; Seattle, WA, USA) and a hydrophone (C54XRS; Cetacean Research Technology; Seattle, WA, USA) and sampled at a rate of 44,100 Hz. The hydrophone was suspended 10 m from shore in the middle of the water column (~ 0.5 m depth) from a stand made from PVC pipe. We piloted each PWC or boat within 1 m of the hydrophone during recordings. We created spectrums of the recordings using the computer program SpectraPRO 3.32 (Cetacean Research Technology; Seattle, WA, USA) and compared these spectrums to the underwater terrapin hearing audiograms.

Results

We obtained a sample AEP series from a female terrapin during exposure to 400 Hz tones at various SPLs (Fig. 4-1). The fast Fourier transforms (FFTs) showed a prominent peak at 800 Hz that occurred within the first 20 ms following onset of the stimulus. We observed the frequency doubling signature in the AEP waveforms which has been found in other aquatic species, i.e., the electrophysiological response appeared at twice the frequency of the stimulus. As stimulus SPL increased, peak amplitude increased and peak latency decreased. For this individual, threshold was 52 dB SPL re 20 μ Pa.

In air, we found that terrapins responded to low-frequency sounds from 50 to 1,600 Hz (Fig. 4-2). The range of best hearing was from 200 to 600 Hz with mean lowest threshold of 50 dB SPL re 20 μ Pa. Male and female audiograms were similar in size, shape, frequency range, and threshold. No significant difference was found between hearing capability of male and female terrapins ($p > 0.05$).

Underwater, four female terrapins responded to sounds from 50 to 800 Hz and one terrapin only responded to sounds from 50 to 400 Hz (Fig. 4-3). The range of best hearing was from 200 to 300 Hz with mean lowest threshold of 86 dB SPL re 1 μ Pa. Terrapins did not respond to sounds greater than 800 Hz, even though they were exposed to 1000 (mean maximum SPL of 126 dB re 1 μ Pa), 1200 (138 dB re 1 μ Pa), and 1600 Hz (131 dB re 1 μ Pa) sounds.

The PWC and recreational boats in Barnegat Bay produced underwater sounds within the hearing range of terrapins (Fig. 4-4). Mean lowest threshold of terrapin underwater hearing was 86 dB SPL re 1 μ Pa and all PWC and boat recordings had higher SPLs suggesting that boat sounds are within the terrapin hearing range. The Parker Boat

had the highest SPL (131 dB re 1 μ Pa), closely followed by the PWC which had a SPL of 121 dB re 1 μ Pa. The Lowe Boat and Polar Kraft, which were the smallest boats with the smallest engines traveling at the slowest speed, had the lowest SPLs. Ambient sound recording had a SPL of 67 dB re 1 μ Pa and all PWC and boat recordings had significantly higher SPLs ($p < 0.05$).

Discussion

We observed the frequency doubling signature in our electrophysiological recordings (Fig. 4-1). When a tone was presented to a terrapin, the electrophysiological response appeared at a frequency twice of the presented tone (e.g., 800 Hz peak when acoustic stimulus was 400 Hz). This phenomenon is likely due to hair cells that are oriented in opposite directions in the saccule which produces two summed evoked potentials in response to an auditory stimulus (Furukawa and Ishii 1967, Sisneros 2007). Frequency doubling is found in many other animals including fish, sharks, and stingrays (Fay 1970, Mann et al. 2001, Casper and Mann 2006).

Terrapins responded to low-frequency sounds in air (50 to 1,600 Hz), as do many other freshwater and marine turtle species (Bartol et al. 1999, Christensen-Dalsgaard et al. 2012). No difference was found in aerial hearing between male and female terrapins; however, only two male terrapins were tested. Terrapins had a narrower range of hearing underwater than in air (Fig. 4-3) which differed from red-eared slider turtles which respond to sounds both in air and underwater from 100 to 1000 Hz (Christensen-Dalsgaard et al. 2012). Red-eared slider turtles respond to 1000 Hz sounds at a mean SPL of 110 dB re 1 μ Pa. We exposed terrapins to 1000 Hz sounds underwater at a maximum mean SPL of 126 dB re 1 μ Pa which should have been high enough to detect a response if one existed.

We found that recreational boats and PWCs in Barnegat Bay produce frequencies and SPLs in the underwater terrapin hearing range. A similar study in the Peconic Bay Estuary system in Long Island, New York found that juvenile sea turtles are exposed to anthropogenic sounds (110 dB re 1 μ Pa rms) during foraging (Samuel et al. 2005). Anthropogenic sounds may negatively affect terrapins and other aquatic organisms in estuaries. The anthropogenic sounds could mask important natural sounds. Although it is unknown if terrapins vocalize, some turtle species such as the long-necked turtle (*Chelodina oblonga*) vocalize during mating (Giles et al. 2009). Anthropogenic sounds could mask conspecific vocalizations and could also mask sounds required to avoid predators or find food.

Behavioral changes could also occur in terrapins due to high levels of underwater anthropogenic sounds. Many terrestrial and marine turtle species change behavior in response to anthropogenic sounds (O'Hara and Wilcox 1990, Lenhardt 1994, Samuel 2004). Desert tortoises (*Gopherus agassizii*) cease movement for up to two hours in response to aircraft noise (Bowles et al. 1999). Loggerhead sea turtles (*Caretta caretta*) avoid areas where seismic air guns are fired (O'Hara and Wilcox 1990, Lenhardt 1994). Kemp's Ridley (*Lepidochelys kempii*) and green (*Chelonia mydas*) sea turtles increase submergence time in response to the sounds from recreational motor boats (Samuel 2004).

Although audiograms created from AEPs is an important first step towards understanding the hearing capability of an organism, future research should focus on determining audiograms based on behavioral methods. AEP audiograms provide an accurate representation of the shape of behavioral audiograms; however, they are much less sensitive (threshold is ~10 to 30 dB higher in AEP audiograms) than audiograms

created by behavioral methods (Borg and Engstrom 1983, Wenstrup 1984, Stapells and Oates 1997). Thus diamondback terrapins may be more impacted by anthropogenic sounds than estimated here. Terrapins may use sound for locating food and mates, avoiding predators, navigation, and communication. More research should be conducted to determine the uses of the sense of hearing for terrapins. The effects of anthropogenic sounds on terrapins both physiologically and behaviorally should also be determined.

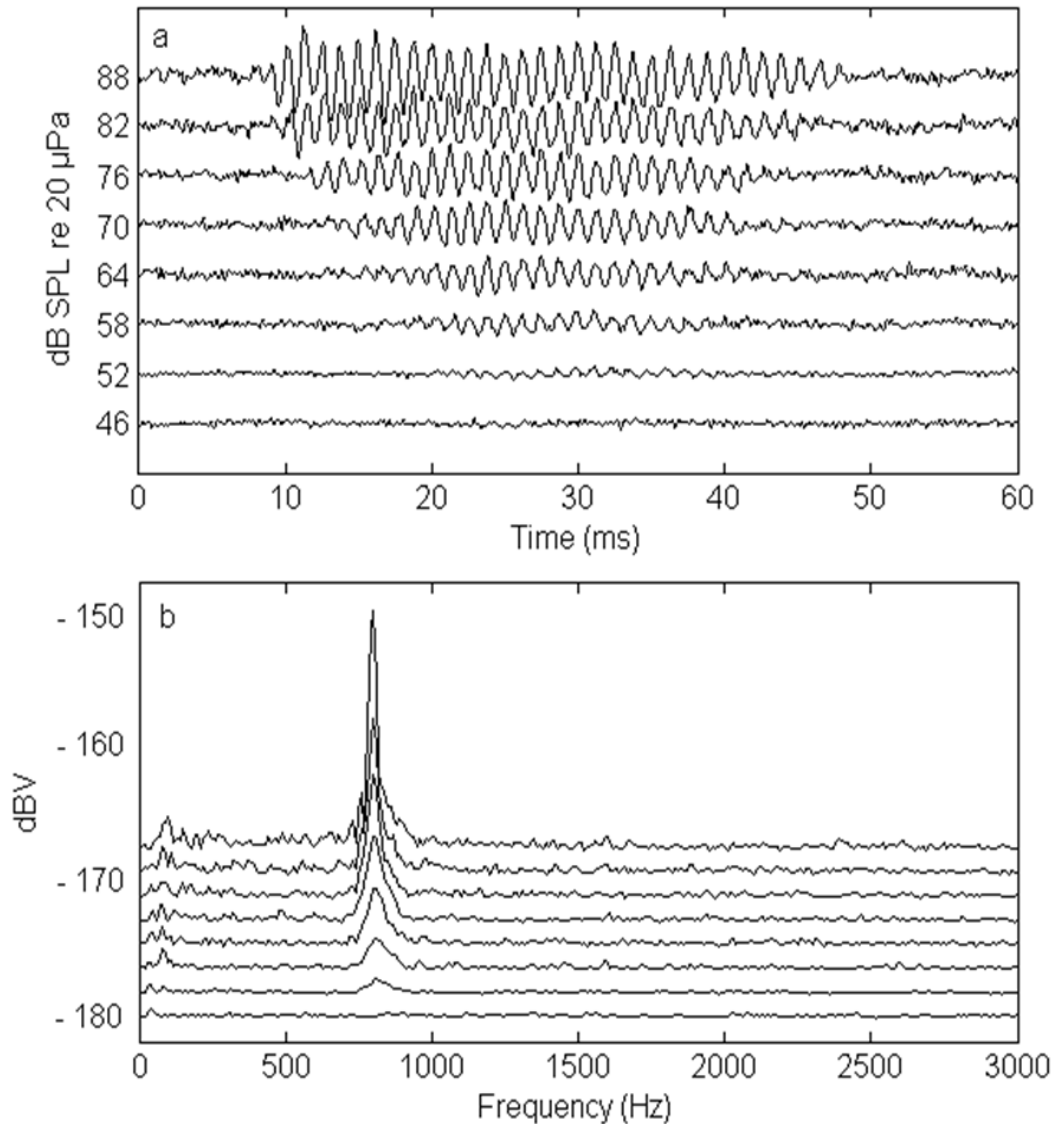


Fig. 4-1. Representative auditory evoked potential (AEP) waveforms. We recorded AEPs from a female terrapin in response to a 400 Hz tone (a). The fast Fourier transform (FFT) of the 400 Hz AEP showed the frequency doubling response at 800 Hz (b). Peak amplitude increased and peak latency decreased as stimulus SPL increased. Terrapin hearing threshold was determined as the lowest SPL where an evoked response was seen in the time domain and/or the FFT (i.e., 52 dB re 20 μ Pa).

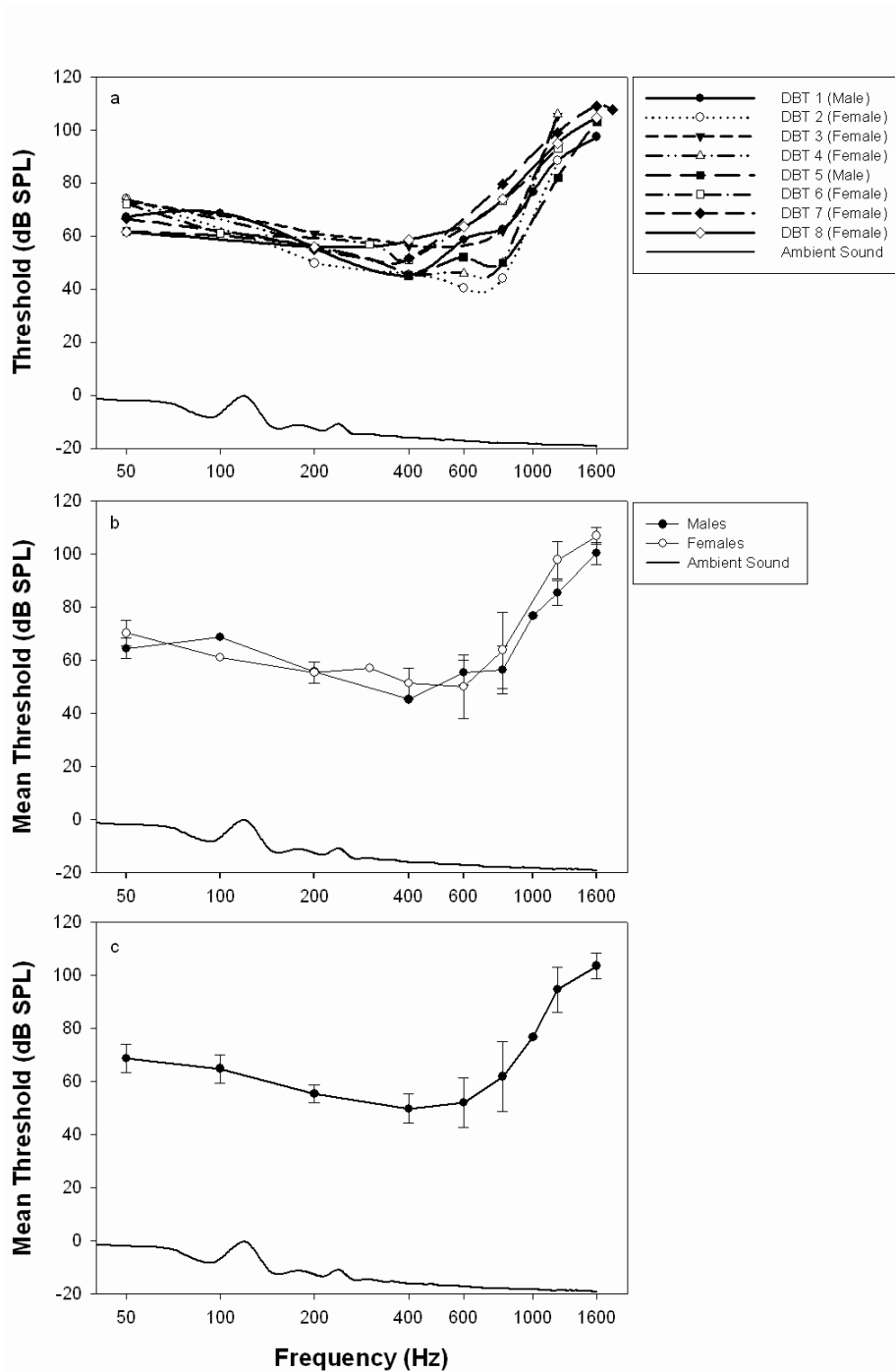


Fig. 4-2. Aerial hearing capability of diamondback terrapins. (a) Auditory evoked potentials were recorded for eight terrapins (two males, six females). (b) No significant difference was found between hearing capability of male and female terrapins. (c) Eight individual audiograms were averaged (error bars are ± 1 standard deviation). Terrapins responded to low-frequency sounds from 50 Hz to 1,600 Hz. The range of best hearing was from 200 to 600 Hz with mean lowest threshold of 50 dB SPL re 20 μ Pa.

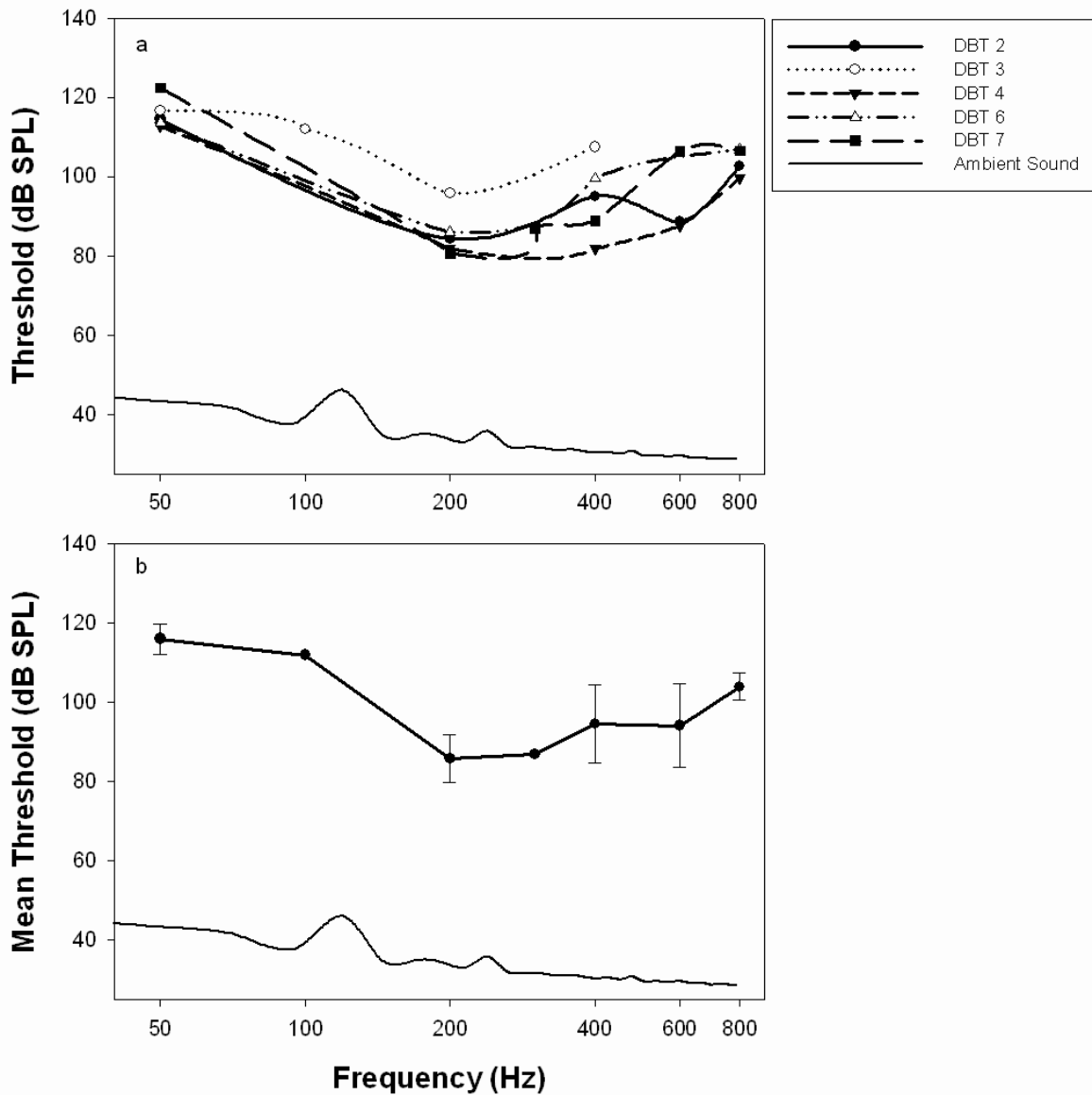


Fig. 4-3. Underwater hearing capability of diamondback terrapins. (a) Underwater hearing capability was tested using the Auditory Evoked Potential technique for five female terrapins. Four of the terrapins responded to low-frequency sounds underwater from 50 to 800 Hz; however, one of the terrapins had a narrower range of 50 to 400 Hz. (b) Individual audiograms were averaged and the range of best underwater hearing was from 200 to 300 Hz with mean lowest threshold of 86 dB SPL re 1 μ Pa. Error bars are ± 1 standard deviation.

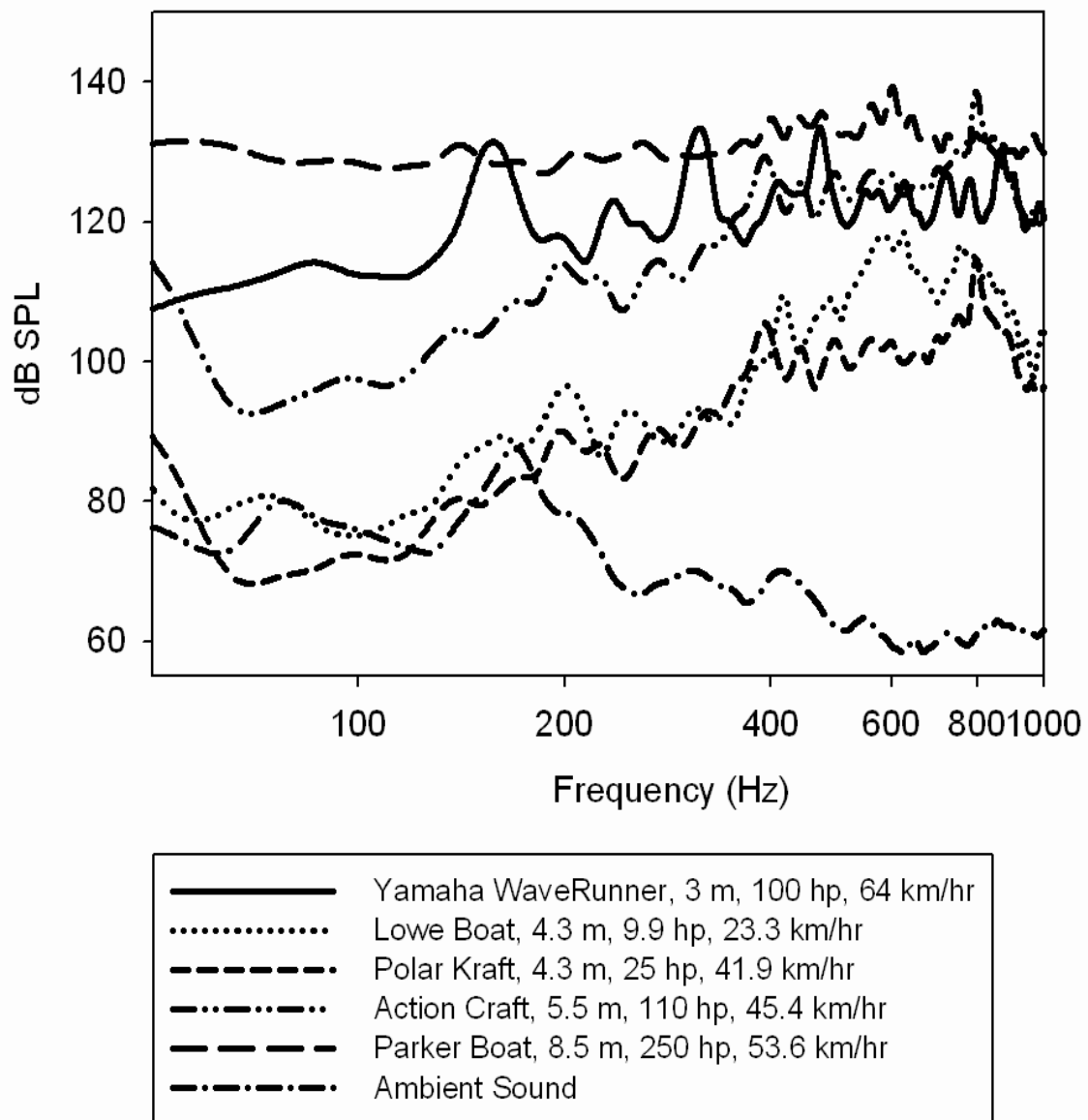


Fig. 4-4. Underwater boat engine sounds in Barnegat Bay, New Jersey, USA.

Recreational boats and personal watercraft (PWC) produce low frequency sounds that are in the hearing range of diamondback terrapins. Female terrapins respond to sounds from 50 to 800 Hz with mean lowest threshold of 86 dB re 1 μ Pa. All boat and PWC recordings showed frequencies above terrapin hearing threshold. Sound pressure level (SPL) was measured in dB re 1 μ Pa.

CHAPTER 5: RECREATIONAL BOATS AND TURTLES: BEHAVIORAL MISMATCHES RESULT IN HIGH RATES OF INJURY

Abstract

Recreational boats are a dominant feature of US estuarine waters. Boat strike injury and mortality may have a detrimental effect on populations of diamondback terrapins (*Malaclemys terrapin*), a keystone species in these ecosystems. In Barnegat Bay, New Jersey, 11% of terrapins ($n = 2,644$) have scars consistent with injuries from boats and injury rates increased from 2006 to 2011. The number of terrapins killed by boat injury is unknown, but may be high. Many boat operators are unaware of the existence of terrapins in the water and terrapins do not respond to the sound of boat motors. When we exposed terrapins to playback recordings of approaching boat engines of varying sizes and speeds *in situ*, terrapins did not significantly change their behavior in response to boat engine sounds. The lack of behavioral response of terrapins to boat sounds and of humans to the presence of terrapins threatens the survival of terrapin populations. Since we cannot change behavior of wild terrapins, the only solution is to change the behavior of humans. A program of boater education combined with partial or complete closure of wildlife areas to boating and strict enforcement of speed limits can protect aquatic wildlife from mortality and injury due to boat propellers.

Introduction

Recreational boating is a popular pastime in the USA where there are over 12 million registered boats (NMMA 2008). Recreational boats may affect aquatic animals directly by causing injury or mortality and indirectly through behavioral or physiological responses to anthropogenic sounds. Many aquatic species are directly affected by

recreational boat propeller strikes including crocodiles (Grant and Lewis 2010), turtles (Bulte et al. 2010), birds (Mikola et al. 1994), and marine mammals (Miksis-Olds et al. 2007). Behavioral and physiological responses of aquatic animals to boat sounds can potentially lead to reduced fitness by lowering survival rates and/or reproductive rates (Southall et al. 2000, Popper 2009).

Many turtle species can hear sounds under 1000 Hz including estuarine (Lester et al. 2012), freshwater (Christensen-Dalsgaard et al. 2012), and sea turtle species (Bartol et al. 1999). Recreational boats produce low-frequency sounds that overlap with turtle hearing ranges (Richardson et al. 1995). Therefore, it is logical to assume that turtles can hear and possibly avoid oncoming boats. However, some diamondback terrapins (*Malaclemys terrapin*, 6 to 20% of the population) have injuries from anthropogenic sources in MD, NC, and SC (Roosenburg 1991, Butler et al. 2006, Hart and McIvor 2008, Cecala et al. 2009). Thus, turtles may not be able to respond quickly enough to avoid boats.

Loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles respond to anthropogenic sounds by increasing submergence time between breaths, spending more time underwater, and swimming to the surface (O'Hara and Wilcox 1990, Samuel 2004). However, these studies on the effects of anthropogenic sounds on sea turtles were performed in laboratory aquaria (Lenhardt 1994, Samuel 2004) where sounds were likely distorted due to reverberation and resonance (Akamatsu et al. 2002). Some marine turtles respond to seismic air guns *in situ* with erratic behavior but these studies are plagued with small sample sizes and individual differences in response (Moein et al. 1994, McCauley et

al. 2000). It remains to be determined if diamondback terrapins respond to the sound of oncoming boats in nature.

The goals of this study were: (1) to measure the rate of terrapin injury due to boat strikes in Barnegat Bay, NJ in order to determine the direct impact of boats on the terrapin population there, and (2) to determine whether terrapins behaviorally respond to boat engine sounds *in situ* in order to understand the role of turtle behavior in this phenomenon. We recorded injuries in terrapins captured over seven years in a mark-recapture population study. Then we used recorded underwater sounds of different sized boat engines to determine the behavioral responses of terrapins to these recordings. Our study was performed *in situ* to better understand how turtles responded to sounds in the natural environment.

Materials and Methods

Study site

This study took place in the Barnegat Division of the Edwin B. Forsythe National Wildlife Refuge (Forsythe) in the Barnegat Bay estuary (Fig. 5-1). Barnegat Bay is a 70 km long estuary located along the eastern coast of New Jersey, USA and is adversely affected by a wide variety of anthropogenic factors, including high levels of recreational boating (BBP 2011).

Field sampling technique

We captured diamondback terrapins using hoop nets, fyke nets, dip nets, and by hand as part of a long term population study of the terrapins in Barnegat Bay. We recorded location of injury including carapace, plastron, bridge, tail, limb, and head. We described shell injuries by writing position and name of broken scutes, and also by drawing injuries

on a diagram of a stereotypical terrapin carapace and plastron. Terrapin injuries were divided into three categories: (1) all injuries, (2) boat injuries, and (3) missing limb. All injuries included carapace, plastron, bridge, tail, limb, head, and boat injuries. Major shell damage (defined as injury to two or more adjacent vertebral, costal, or plastral scutes, or three or more adjacent marginal scutes, Fig. 2-2) in adult turtles is typically caused by anthropogenic sources such as boats (Cecala et al. 2009) and automobiles (Wood and Herlands 1997). We assumed that major shell injuries were from recreational boat propellers because terrapin nesting beaches are not located near roads in Forsythe. In general, diamondback terrapins with boat injuries tend to have slash marks from propellers and those with automobile injuries tend to have crushed shells. However, some diamondback terrapins in Forsythe Refuge appear to be hit by the hull of boat or personal watercraft (PWC) instead of the propeller and display a crushed carapace similar to those injuries seen in terrapins that have been hit by automobiles. We used linear regression to determine if there was a temporal change in injury rates from 2006 to 2011.

Experimental methodology

We exposed small ($n = 40$, 400 to 600 g body mass) and large ($n = 40$, 1,000 to 1,200 g body mass) non-gravid, uninjured female terrapins to playback recordings of approaching boat engines. Female terrapins were selected because they attain significantly larger body size than males allowing total mass of data loggers attached to terrapins to stay below 5% of body mass (Gibbons and Lovich 1990). Non-gravid individuals were selected because they were less likely to exit water to pursue nesting areas. We chose to study two different size classes of terrapins because older (i.e., larger) females may be more

habituated to boat engine sounds, while younger (i.e., smaller) females may have better hearing capability.

Playback recordings

We recorded recreational boat sounds with a digital recording computer (Sound DSA ST 191; Cetacean Research Technology; Seattle, WA, USA) and a hydrophone (C54XRS; Cetacean Research Technology; Seattle, WA, USA) of four different vessels varying in length and outboard motor engine size. Each of the four vessels was piloted at maximum speed past the hydrophone, parallel to the shore. At the closest point, vessels were 1 m from hydrophone. Vessels used were a Lowe boat (Johnson 9.9 hp outboard motor, 4.3 m length, 22.9 km/hr speed), a Polar Kraft boat (Mercury 25 hp outboard motor, 4.3 m length, 41.9 km/hr speed), an Action Craft boat (Johnson 110 hp outboard motor, 5.5 m length, 40 km/hr speed), and a Parker boat (two Johnson 150 hp outboard motors, 8.5 m length, 53.4 km/hr speed). We measured sound spectrum with the computer program SpectraPRO 3.32 (Cetacean Research Technology; Seattle, WA, USA) for each 1 min long recording (Scholik and Yan 2002).

Data loggers and transmitters

We outfitted each terrapin with a HOBO Pendant G acceleration data logger (UA-004-64, accuracy $\pm 2.5^\circ$; Onset Computers; Bourne, MA, USA) and a Data Storage Tag (DST) milli-L temperature and depth data logger (depth range 10 cm to 20 m, depth accuracy ± 8 cm; Star-Oddi; Reykjavik, Iceland; Fig. 5-2). The hobo pendant G data logger recorded x-, y-, and z-axis orientation of the terrapin in degrees every 1 s and the DST recorded depth and temperature every 1 s during the trials. We calculated mean change in

pitch (x-axis) and roll (y-axis) of terrapins before, during, and after sound by taking the mean of the absolute values of each value (°) minus the value from 1 s previous.

A sonic transmitter (IBT-96-5; 8.5 g; Sonotronics; Tucson, AZ, USA) and a radio transmitter (V2B154, two-stage, 164 MHz, 27 g; Sirtrak; Havelock North, New Zealand) allowed us to relocate the terrapin if it left the study site (Fig. 5-2). We detected sonic transmitters using a submersible sonic receiver (N15A235B; Dukane Underwater Acoustic Location Receiver; St. Charles, IL, USA) and radio signals were detected with a two-element yagi antenna connected to a radio receiver (R1000; Communications Specialists Incorporated; Orange, CA, USA).

Experimental design

We exposed each terrapin to playback recordings in a 60 m segment of a canal (locally called a mosquito ditch) that was approximately 1.5 m deep by 2 m wide located in Forsythe (Fig. 5-1). Mosquito ditches are straight, narrow canals that were dug to control mosquito populations. We blocked off both ends of the mosquito ditch with plywood to ensure that terrapins were contained in the arena and tidal water flow that could otherwise influence behavior was minimized. All trials took place within two hours of high tide to ensure the arena had maximum water depth (~ 1.5 m).

An underwater speaker (LL9816; Lubell Labs; Columbus, OH, USA) suspended at a depth of 75 cm at the midpoint of the 60 m long arena and connected to a Speco Amplifier (PAT 20 TB 20 Watt 12 V PA; Speco Technologies; Amityville, NY, USA) broadcast boat motor sounds played with the computer program, SpectraPRO 3.32. We monitored playback recordings prior to each trial with the hydrophone (C54XRS)

suspended at various distances (1 m, 5 m, and 10 m) from the underwater speaker to determine sound propagation in the canal.

We initiated each trial by releasing a terrapin into the water at one end of the canal (randomized). The terrapin swam freely and when it was within 10 m from the speaker, we started playback recording. Each trial ended when the terrapin completed swimming 60 m total. We determined swimming speed by measuring how long each terrapin spent swimming in each 10 m long section. We standardized swim speed as a function of the body length of each terrapin using straight carapace length expressed as body lengths s^{-1} .

Data analyses

We performed a power analysis prior to experimentation to ensure adequate sample size. Our power test showed that a sample size of eight individuals per treatment was necessary for behavior trials in response to recorded boat sound for a power of 0.8. We had a sample size of ten individuals ($n=10$) of each size class (small and large) for each of the four boat engine recordings for a total of 80 individuals. Our sample size of 10 had a power of 0.96.

We used a multivariate mixed-effects model to test for significant differences before, during, and after exposure to playback recordings. Fixed effects included treatment (sound or control), terrapin size (small or large), and time (before, during, or after sound playback). Random effects included individual terrapins ($n = 10$) and number of trials (3 trials per terrapin).

Results

Many terrapins sustained boat injuries in Edwin B. Forsythe Wildlife Refuge (Fig. 5-3; $n = 291$ of 2,644). Mean all injury ($Y = 0.02 X - 48.02$, $R^2 = 0.68$, $P = 0.04$) and boat

injury ($Y = 0.01 X - 22.8$, $R^2 = 0.82$, $P = 0.01$) rates of terrapins increased temporally from 2006 to 2011 with an overall mean boat injury rate of 0.11. The 11% injury rate was an indication of terrapins that survived injury to capture and not total injury rate. We do not know how many terrapins were killed by impacts with boats or propellers because many of those terrapins probably sank to the bottom or were consumed by scavengers. Three dead terrapins were found in 2011 washed up on Conklin Beach which is the major nesting site for terrapins in the Forsythe Refuge. All three dead terrapins had major carapace and plastron damage due to boat propeller strikes and one terrapin also had major head and neck injuries. The mean frequency of missing limbs in terrapins in Forsythe did not increase temporally from 2006 to 2011 ($Y = 0.009 X - 17.2$, $R^2 = 0.32$, $P = 0.24$).

The majority of boats we saw in the creeks of Forsythe Refuge were personal watercrafts (PWC), small recreational boats up to 7.6 m with motors up to 150 hp, and small commercial crabbing vessels about 6 m. Many boaters speed through the creeks despite posted speed limits and could easily hit turtles considering how shallow the water was in the creeks (commonly less than 0.5 m deep during low tide). Many local boat operators are not aware that terrapins are found in the bay. Terrapins also travel in the open waters of Barnegat Bay (Sheridan 2010) and there they are exposed to hundreds of vessels ranging in size from 3.7 m sail boats to 9.1 m speed boats with large high powered engines that propel the boats at more than 45 mph (known locally as cigarette boats). The potential for injury to terrapins was likely very high in both environments.

Boat engine sounds in Barnegat Bay were in the hearing range of terrapins (Lester et al. 2012) with low-frequency components with maximum sound pressure levels (SPL) between 100 and 140 dB re 1 μ Pa rms in the 400 to 600 Hz range (Fig. 5-4). When we

measured spectrums of the playback recordings at 1 m from the underwater speaker, playback boat sounds had SPLs that were lower than the corresponding original recording but were still in the hearing range of diamondback terrapins (Fig. 5-5). At 1 m from speaker, mean SPL was 15 dB re 1 μ Pa rms lower than original recording for Lowe Boat, 18 dB re 1 μ Pa rms for Polar Kraft, 28 re 1 μ Pa rms for Action Craft, and 20 re 1 μ Pa rms for Parker Boat. Playback boat sound was not detectable from ambient sound in the ditch by 10 m from underwater speaker.

Terrapins did not significantly change behavior in response to playback recordings (Table 5-1). Swimming speed did not differ before, during, or after playback recordings (p-values ranged from 0.14 to 0.76). There were no significant differences in swimming speeds (p-values ranged from 0.09 to 0.89) due to sound exposure. Swimming depth did not significantly change in response to exposure to acoustic recordings of approaching boat engines (p-values ranged from 0.21 to 0.81). Mean depth of terrapins varied from 0.1 m to 0.25 m. Pitch and roll varied from 3° to 5°. There were no significant differences in mean absolute value of change in pitch or roll in response to playback recordings in either size class (p-values ranged from 0.07 to 0.70 for pitch and 0.09 to 0.73 for roll) indicating that terrapins were not making sudden or erratic movements in response to boat sounds.

Discussion

Terrapins did not significantly alter their behavior in response to playback recordings of approaching boat engines (Table 5-1). The result was a high rate of boat injury to surviving terrapins (11%) and an unknown, but likely high rate of death of turtles impacted by boats. Anthropogenic mortality has an important negative impact on both freshwater turtles (Steen and Gibbs 2004) and sea turtles (Spotila et al. 2000) and can drive

populations towards extinction (Saba et al. 2012). Therefore, we need to understand the basis for the negative interactions of boats on terrapins in Barnegat Bay in order to develop a solution to sustain that population.

McGregor (2000) identified three reasons that significant differences may not be found in behavior during playback experiments. First, the subjects may not be able to perceive the difference between control and experimental exposure. Terrapins physiologically respond to low-frequency sounds less than 1000 Hz (Lester et al. 2012). Recreational boat engines in Barnegat Bay produced low-frequency sounds within this hearing range of terrapins. Second, the variables measured may not be sensitive enough to detect a significant behavioral response (McGregor 2000). The terrapin's depth and orientation were recorded every 1 s during the trials which was sensitive enough to detect a response. Swimming speed was measured in each 10 m segment of the experimental canal through which the terrapin swam. It is possible that this variable was not sensitive enough to detect a startle response because terrapins may increase their speed for smaller distances. However, the depth and orientation changes would have been recorded. Third, the animals may be able to detect the difference but their behavioral response may be the same regardless of whether or not the sound is played. Because terrapins in Barnegat Bay are exposed to high levels of recreational boating traffic (MTA 2008), those used in this study may have been habituated to the sounds produced by boat engines and therefore may not respond behaviorally. Further testing using naïve terrapins may determine whether this was the case. Loggerhead and green sea turtles do not behaviorally respond to anthropogenic sounds unless the SPL of the sound exceeds 166 dB re 1 μ Pa rms (McCauley et al. 2000). We did not expose diamondback terrapins to boat sounds with SPLs as high as those used

by McCauley et al., but we also did not find boat sounds with SPLs that high in Barnegat Bay. Nevertheless, diamondback terrapins that live in Barnegat Bay do not respond to boat engine sounds at SPLs that are present in their environment and thus are at risk of injury.

Sound in water is composed of pressure waves and particle motion (Fay and Popper 1975). Particle motion is the main mode of hearing for many fish species because fish otoliths can directly detect particle motion components of sound due to inertial differences between otoliths and sensory epithelia (Popper and Fay 2011). Although diamondback terrapins did not respond behaviorally to playback recordings of boat engines, we only exposed terrapins to pressure components of boat engine sounds. Future studies should measure diamondback terrapin sensitivity to acoustic particle motion. Turtles may also use a combination of pressure hearing and particle motion detection.

Turtles may use cues other than sound to alert them to oncoming anthropogenic disturbances such as boats. For example, terrapins may be able to determine a boat is approaching by the shadow cast into the water by the vessel or the displacement of the water by the approaching boat. Harrison (2010) found that medium (833 to 895 g) and large (1067 to 1170 g) female terrapins increased their depth by 0.16 to 0.18 m ($p < 0.05$) when in the presence of a moving boat. However, it is unlikely that the shadow cast by a quickly approaching boat would give a terrapin enough warning prior to impact. A terrapin may have enough time to dive under the boat to avoid direct impact but may then get pulled into the propeller. Green sea turtles can avoid boat propellers if the boat is traveling at less than 4 km hr^{-1} but the proportion of turtles that can avoid vessels decreases significantly as speed increases (Hazel et al. 2007).

Many boat operators in Barnegat Bay do not respond to the presence of terrapins. Boaters speeding through the narrow creeks of the Forsythe Wildlife Refuge on a jet ski or in a power boat do not look for turtles. Many boaters do not even know that terrapins are present in the Bay. Turtles have little opportunity to get out of the way of oncoming boats. In the open waters of the Bay, even boaters moving at moderate speed cannot see a turtle in the turbid water ahead.

The lack of behavioral response of terrapins to the sound of approaching boats and the lack of behavioral response of boat operators to terrapins explains the high rate of injury to terrapins due to boat strikes in Barnegat Bay. In the Forsythe Refuge, 11.0% of terrapins have boat injuries (Fig. 5-3). These injury rates are likely an underestimate of the actual proportion of the terrapin population to be affected by boat propeller strikes. Fishery interactions with various marine turtle species often lead to sea turtle deaths with the carcass ending up at the bottom of the water column (Crowder et al. 1995, Epperly et al. 1996). The same is likely true for terrapins since we do not find many carcasses due to fatal collisions. Thus the actual rate of boat collision is unknown and most likely greater than observed in most studies.

Many diamondback terrapin populations are threatened by various anthropogenic factors including drowning in crab pots, being hit by automobiles while searching for nesting habitat, and being preyed upon by non-native predators (Bishop et al. 1983, Wood and Herlands 1997, Draud et al. 2004). The impacts of boat injury and mortality of aquatic turtles add an additional serious threat to survival of terrapin populations. Action is needed to reduce and eliminate this controllable source of mortality.

Since terrapins do not react to boat sounds and have limited ability to avoid collisions with fast approaching boats, the solution to this problem lies with the human component of the interaction. Humans can learn and change their behavior through a combination of education and regulations. Since all US states with terrapin populations require a boater education course to obtain licenses to operate a power boat, information could be included on the ecology and behavior of terrapins and other aquatic wildlife during these courses. This educational component could succeed if reinforced with regulations on speed limits and area closures of terrapin habitat. Partial or complete closures of wildlife areas during high activity seasons of mating, nesting, and foraging are necessary to protect individuals. In Barnegat Bay, partial closures of boating areas by nesting beaches are necessary during nesting season (from the end of May to the middle of July). Furthermore, speed limits should be implemented and enforced in areas of high turtle density. Implementing regulations regarding boat use in habitats with high turtle densities will be beneficial for conserving turtle populations worldwide.

Table 5-1. Behavioral responses to playback recordings of approaching boats. Small (400 to 600 g) and large (1000 to 1200 g) terrapins (n = 80) were exposed to playback recordings of approaching recreational boats during sound trials and no sound during control trials. Behavioral response of terrapins to playback recordings was not significantly different from behaviors before (B), during (D), or after (A) sound exposure (mixed-effects models: $p > 0.05$).

Motor Size (hp)	Trial Type	Body Size	Mean Swimming Speed (body length / s)						Mean Swimming Depth (m)								Mean Change in Pitch (°)			Mean Change in Roll (°)			Mixed Effects Model
			Distance from Speaker (m)						Time (min)								Time			Time			
			25	15	5	5	15	25	15	30	45	60	75	90	105	120	B	D	A	B	D	A	
9.9	Sound	Large	1.68	1.69	1.90	2.22	1.97	1.80	0.16	0.16	0.13	0.13	0.12	0.13	0.14	0.15	5.69	6.15	5.49	6.67	6.22	5.97	n.s.
	Control	Large	1.60	1.88	1.87	2.08	1.95	1.65	0.14	0.13	0.14	0.13	0.13	0.15	0.15	0.14	3.62	3.06	3.55	3.44	3.30	3.58	n.s.
	Sound	Small	1.61	1.79	1.84	2.13	1.89	1.83	0.12	0.13	0.14	0.12	0.11	0.14	0.13	0.14	4.77	5.32	5.54	5.59	6.37	6.24	n.s.
	Control	Small	1.74	1.93	1.77	1.91	1.74	1.67	0.11	0.13	0.13	0.12	0.12	0.14	0.13	0.13	3.67	3.28	3.55	3.95	3.34	3.31	n.s.
25	Sound	Large	1.64	1.60	1.89	1.93	1.88	1.73	0.23	0.24	0.23	0.24	0.23	0.23	0.24	0.23	3.45	3.53	3.96	3.51	3.55	3.72	n.s.
	Control	Large	1.81	1.87	1.79	1.80	1.77	1.63	0.23	0.23	0.21	0.21	0.23	0.22	0.23	0.23	3.37	3.55	3.97	3.29	3.31	3.32	n.s.
	Sound	Small	1.79	1.66	1.79	1.77	1.77	1.66	0.21	0.21	0.22	0.22	0.22	0.23	0.22	0.23	3.69	3.64	4.40	3.82	3.84	3.87	n.s.
	Control	Small	1.78	1.73	1.80	1.85	1.84	1.79	0.24	0.23	0.23	0.22	0.22	0.23	0.25	0.23	4.15	3.78	3.84	4.27	4.17	3.64	n.s.
110	Sound	Large	1.91	1.80	2.02	2.00	1.93	1.82	0.24	0.25	0.25	0.24	0.24	0.25	0.24	0.25	3.62	3.85	4.08	3.94	3.73	4.01	n.s.
	Control	Large	2.00	1.84	1.83	1.84	1.89	1.77	0.24	0.25	0.25	0.24	0.25	0.24	0.23	0.24	3.89	3.82	4.04	4.22	3.83	3.55	n.s.
	Sound	Small	1.84	1.72	2.23	1.82	1.59	1.67	0.21	0.22	0.23	0.22	0.20	0.21	0.23	0.22	4.11	4.00	4.88	4.04	3.87	4.40	n.s.
	Control	Small	1.85	1.68	1.81	1.76	1.72	1.68	0.20	0.19	0.21	0.22	0.20	0.21	0.20	0.19	4.29	4.02	4.70	4.63	3.73	3.88	n.s.
250	Sound	Large	1.61	1.69	1.70	1.86	1.78	1.63	0.18	0.18	0.17	0.18	0.16	0.17	0.19	0.17	4.27	4.58	4.46	4.87	4.81	4.42	n.s.
	Control	Large	1.56	1.64	1.61	1.73	1.89	1.52	0.18	0.19	0.17	0.18	0.17	0.15	0.18	0.17	3.13	3.39	2.98	3.36	3.20	3.08	n.s.
	Sound	Small	1.70	1.70	1.87	1.88	1.67	1.73	0.15	0.15	0.16	0.14	0.16	0.15	0.14	0.16	4.02	4.58	4.28	4.34	4.00	4.35	n.s.
	Control	Small	1.62	1.70	1.63	1.81	1.66	1.76	0.15	0.15	0.16	0.15	0.17	0.15	0.15	0.15	4.06	4.30	4.24	3.79	3.82	3.79	n.s.

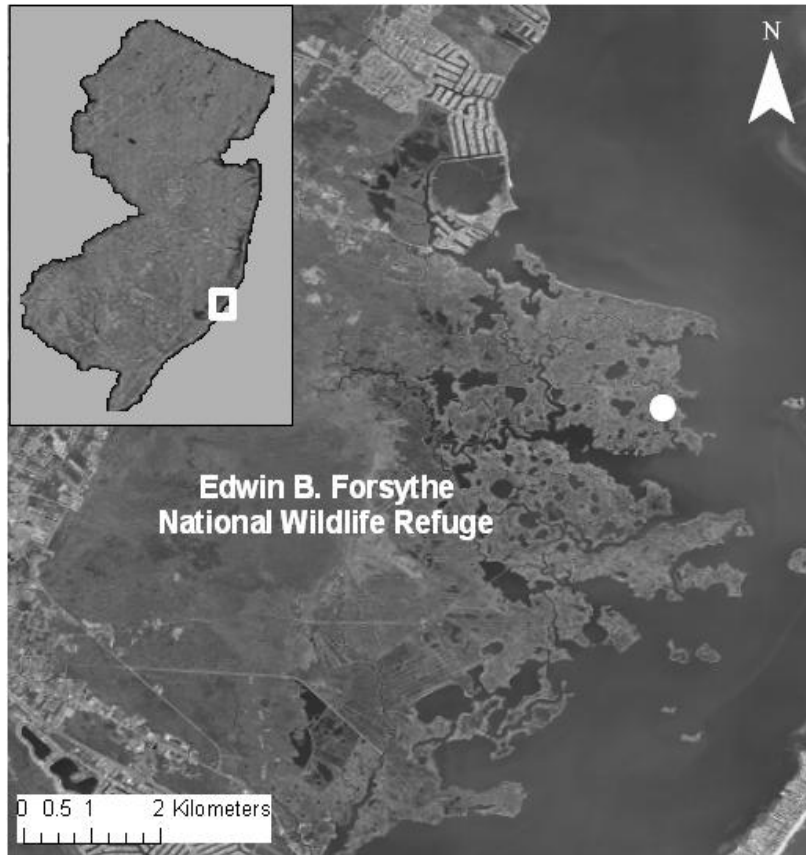


Fig. 5-1. Study site map. Terrapins were collected in the Edwin B. Forsythe Wildlife Refuge in Barnegat Bay, New Jersey, USA. Playback trials were conducted in a canal (mosquito ditch) designated with a white circle on the map.

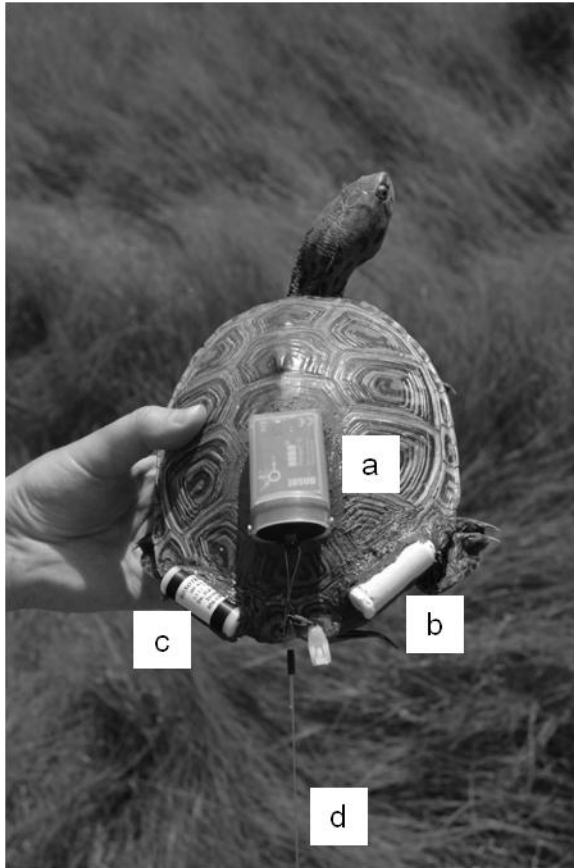


Fig. 5-2. Data loggers and transmitters attached to an adult female diamondback terrapin. A HOBO Pendant G data logger recorded the orientation of the terrapin in the water every 1 s (a). A Data Storage Tag milli-L temperature and depth data logger recorded depth of the terrapin every 1 s (b). Sonic (c) and radio (d) transmitters allowed us to relocate terrapin in case of escape. Transmitters and data loggers weighed < 5% body mass of terrapins.

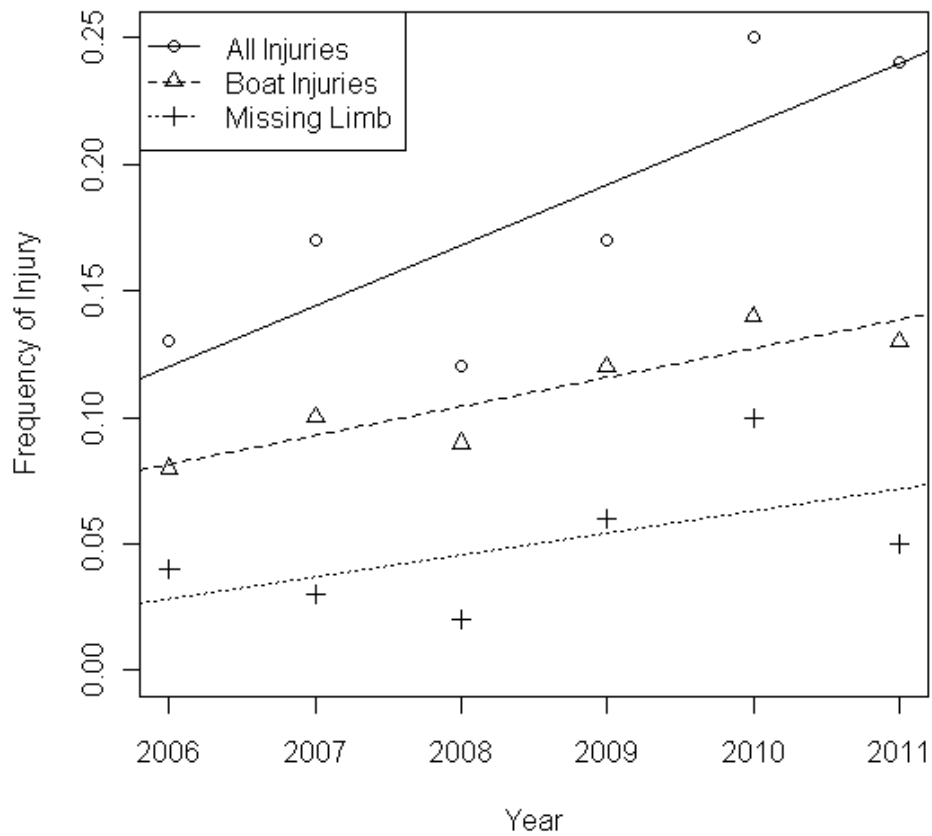


Fig. 5-3. Injury rates of diamondback terrapins in Barnegat Bay, New Jersey. All injuries ($Y = 0.02 X - 48.02$, $R^2 = 0.68$, $P = 0.04$) and boat injuries ($Y = 0.01 X - 22.8$, $R^2 = 0.82$, $P = 0.01$) of terrapins increased temporally from 2006 to 2011. Mean terrapin boat injury rate was 0.11. Frequency of missing limb in terrapins did not increase temporally from 2006 to 2011 ($Y = 0.009 X - 17.2$, $R^2 = 0.32$, $P = 0.24$). Number of boat injuries resulting in death was unknown because dead animals were lost to the natural system.

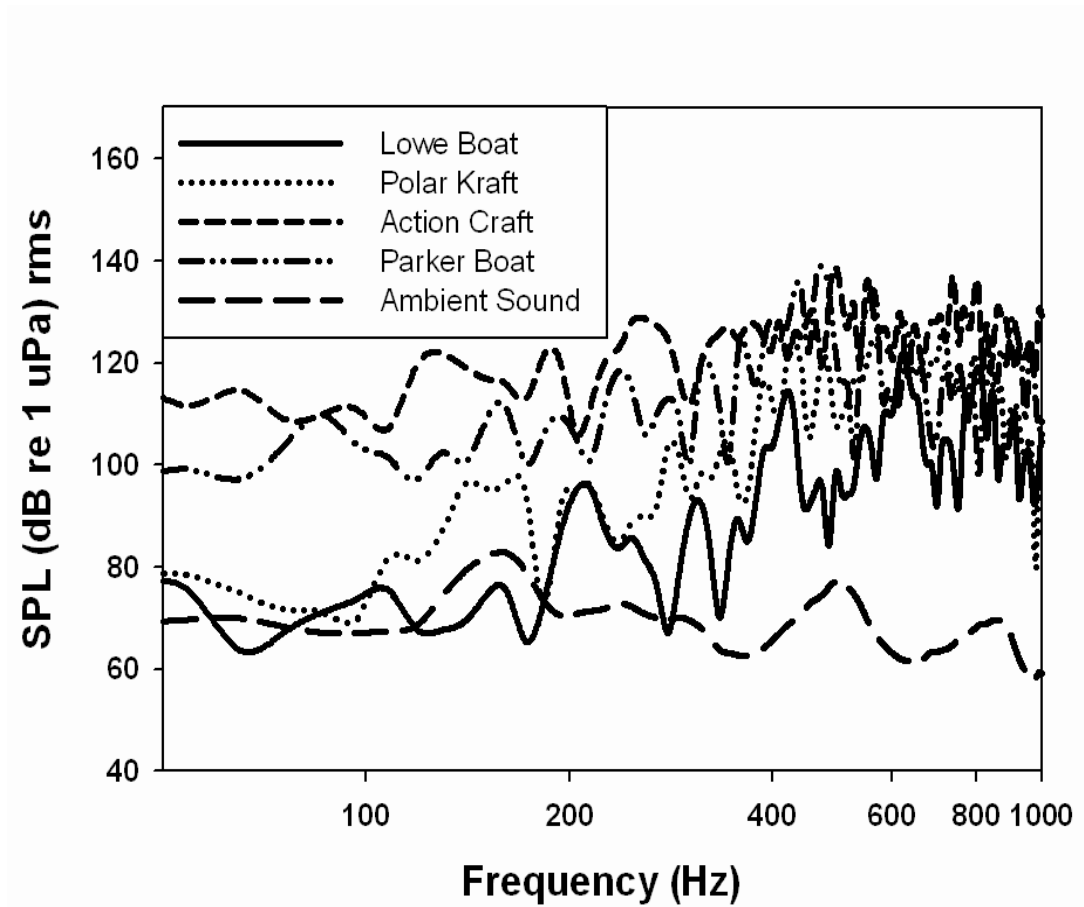


Fig. 5-4. Mean boat spectrums. Each terrapin was exposed to one of four different boat engine recordings. The maximum sound pressure level varied from 100 to 140 dB re 1 μ Pa in the 400 to 600 Hz range. The range of best hearing for terrapins (i.e., the frequencies at which terrapins can hear the lowest thresholds) is also from 400 to 600 Hz.

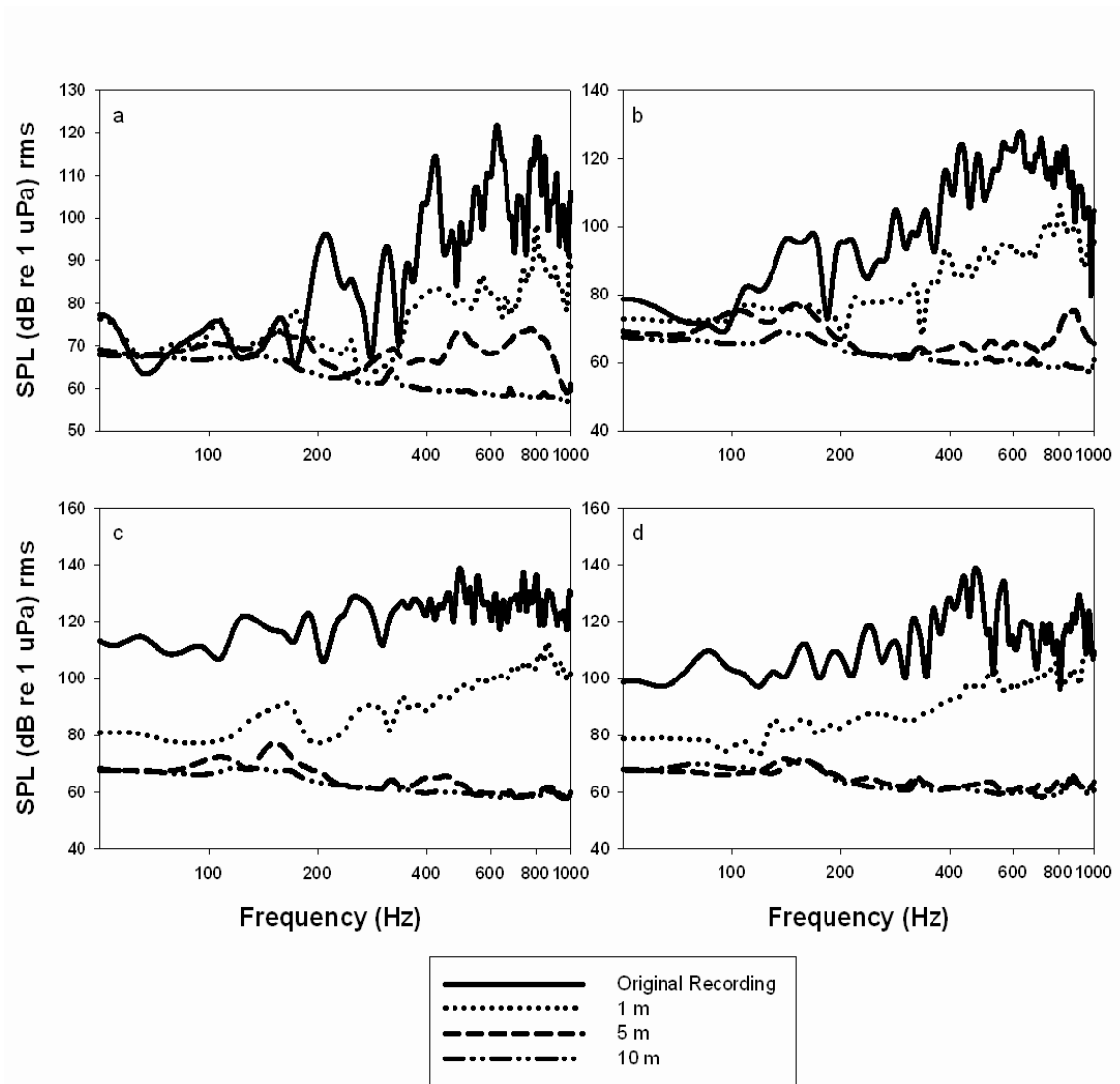


Fig. 5-5. Sound propagation in the experimental canal. We measured mean spectrums of each boat recording (a – Lowe Boat, b – Polar Kraft, c – Action Craft, d – Parker Boat) at various distances (1 m, 5 m, and 10 m) from the underwater speaker. At 1 m from the speaker, the playback boat sound was a mean SPL of 15 to 28 dB re 1 μ Pa less than original recording depending on which boat sound was playing. At 10 m from speaker, the playback boat sound was not detectable from ambient sound in the ditch.

CHAPTER 6: CONSERVATION AND MANAGEMENT SUGGESTIONS

Dissertation Summary

Injury Rates

Many terrapins in Barnegat Bay, New Jersey have scars that are consistent with injuries from anthropogenic sources such as recreational boats and automobiles. Frequency of anthropogenic injuries to terrapins (mean, 0.11 to 0.15) in Edwin B. Forsythe National Wildlife Refuge (Forsythe) and Island Beach State Park (IBSP) increased temporally from 2006 to 2011. This increase may correspond with more recreational boats being present in Barnegat Bay (MTA 2008). Larger terrapins were more likely to exhibit anthropogenic injuries than small terrapins with the exception of male terrapins in Forsythe. Male terrapins in Forsythe and female terrapins in Island Beach State Park (IBSP) with anthropogenic injuries had significantly lower survivorship than uninjured terrapins.

Hearing

Hearing capability of terrapins was determined using two different techniques. In Chapter 3, auditory evoked potentials were recorded from five terrapins with a closed-coupler and the acoustic stimulus alternated between clicks and clicks plus an added tonal marker. Terrapins responded to in-air sounds from 100 to 1000 Hz and range of best hearing was from 400 to 600 Hz with mean lowest threshold of 64 dB re 20 μ Pa SPL (sound pressure level). Resonance frequency was calculated from CT scans of two terrapins' heads. The male terrapin had a resonance frequency of 900 Hz and the female terrapin had a resonance frequency of 600 Hz. Underwater hearing capability of terrapins was estimated from CT scans, in-air audiogram, and previous research on red-eared slider

turtles (Christensen-Dalsgaard et al. 2012). Female terrapins were predicted to hear best underwater from 300 to 500 Hz with lowest threshold of 75 dB re 1 μ Pa SPL. Male terrapins were estimated to have a best frequency underwater hearing range of 200 to 400 Hz with lowest threshold of 70 dB re 1 μ Pa SPL.

In Chapter 4, auditory evoked potentials (AEP) were measured from diamondback terrapins in-air and underwater. Terrapins responded to stimuli from 50 to 1600 Hz in-air, with the range of best hearing from 200 to 600 Hz with mean lowest threshold of 50 dB re 20 μ Pa SPL. Underwater, terrapins responded to tones from 50 to 800 Hz and range of best hearing was from 200 to 300 Hz with mean lowest threshold of 86 dB re 1 μ Pa SPL. Recreational boat engines produce sounds that overlap with the underwater hearing range of terrapins.

Behavioral Response to Boat Sounds

In Chapter 5, terrapins were exposed to playback recordings of approaching recreational motor boats of varying sizes and speeds *in situ*. Terrapins did not behaviorally respond to boat sounds by changing swimming speed, swimming depth, or body orientation in the water. This lack of behavioral response to boat sounds may explain high injury rates of terrapins found in Barnegat Bay, NJ. Since diamondback terrapins do not avoid approaching recreational boats, human behavior must change to ensure that terrapin populations are maintained.

Future Considerations

Injury Rates

Further research is necessary regarding the cause of injuries in terrapins in order to determine effective prevention methods. Mark-recapture studies are essential in

various locations to assess terrapin injury rates. Terrapin injury rates have been assessed in MD (Roosenburg 1991), SC (Cecala et al. 2009), and FL (Hart and McIvor 2008); however, injury rate analyses should be conducted in other areas along the East and Gulf coasts of the USA. Estimates of potential boat related mortality are also necessary considering that injury rates are underestimates of the impact of boats on terrapin populations. Boat speed and collision risk should also be assessed to determine whether speed limits would be beneficial to decreasing terrapin injury and mortality rates.

Hearing

Although audiograms acquired through electrophysiological techniques provide accurate audiogram shape, hearing threshold is commonly 10 to 30 dB lower when behavioral methods are utilized (Gorga et al. 1988, Brittan-Powell et al. 2002). Thus behavioral studies should be conducted to determine hearing thresholds of terrapins. Furthermore, pressure sensitivity was measured in terrapins, but particle motion was not measured. Some aquatic animals including many fish species use a continuum of particle motion and pressure sensitivity to detect sounds (Popper and Fay 2011). Pressure sensitivity tends to be a result of air bubbles located near the ear, and many turtle species have air-filled middle ears. However, particle motion may be responsible for some sound detection in terrapins. More research is necessary regarding diamondback terrapin hearing use to determine the purpose of the sense of hearing for terrapins. Hearing may be used to locate food or mates, avoid predators, navigate, or communicate.

We created audiograms for captive terrapins in air and underwater and measured spectrums of boat engine sounds in Barnegat Bay. Although the boat engine sounds were within the hearing range of captive terrapins, we did not use boat engine sounds as an

acoustic stimulus for measuring auditory evoked potentials (AEPs) of wild terrapins. Further research should measure AEPs of wild caught terrapins in response to boat engine sounds to determine if wild terrapins can detect the stimulus.

Behavioral Response to Boat Sounds

Although we found no behavioral response of terrapins to playback recordings of approaching boat engines, we did not monitor physiological responses. Future research should measure physiological responses such as stress hormones and heart rate before, during, and after anthropogenic sound exposure. Gopher tortoises decrease heart rate by 7.6% in response to jet aircraft sounds (Bowles et al. 1999). Furthermore, we did not measure acoustic stimulus as received by the terrapins instead we measured sound propagation through the experimental canal. Sound recording tags should be attached to terrapins in future studies to allow researchers to know the received sound pressure levels at all times during trials.

Management Recommendations

Recreational boating will likely continue to increase over time in diamondback terrapin-rich habitats (NMMA 2008). In order to prevent terrapin boat injury rates from increasing further and negatively impacting populations, rules and regulations regarding boat use must be implemented. Watercraft activity, including recreational boats and personal watercraft, will need to be limited in high risk areas of the bay at certain times. Boating activity should be limited in critical habitats or aggregation areas for diamondback terrapins including nesting, brumating, foraging, and basking sites. Terrapin nesting season varies depending on area, but in Barnegat Bay nesting season extends from mid-May to the end of July. Conklin Island is the major nesting beach for

diamondback terrapins in Forsythe Refuge. From mid-May to the end of July, boat use should be restricted in the waterway directly in front of Conklin Island's nesting beach. Furthermore, diamondback terrapins tend to commonly utilize waterways surrounding estuarine emergent wetland (Sheridan 2010). In these areas, terrapins are prone to boat injuries due to the shallow water. During low tide, many boat propellers are dragged through the mud at the bottom of the creeks where terrapins are prone to hide. Recreational boating should be limited in these small tidal creeks, especially during low to mid tide when the water is especially shallow (oftentimes less than 0.5 m deep). In addition to diamondback terrapins, restricting boat use could be beneficial to other aquatic organisms including game fish species.

If restricting boat use is not an option, restrictions include speed limits and limits on engine size of boats. Speed limits may allow terrapins and other wildlife more time to evade injury from approaching boats. Speed limits have been successful for preventing boat injuries to manatees in Florida, USA (Reynolds 1999, Aipanjiguly et al. 2003). Limiting engine size would also be beneficial by reducing maximum speed possible by vessels and decreasing depth of propeller in water. Both speed and engine size limits will only be successful in lowering injury rates if well publicized and strictly enforced.

Finally, the public needs to be provided with educational materials regarding the ecology of diamondback terrapins and other wildlife species and the rules and regulations that exist to reduce injury rates to terrapins. In particular, boaters, anglers, and waterfront landowners should be provided with educational materials identifying species of special concern and reviewing regulations on recreational boating. All east and gulf coast states in the USA require that recreational boaters take a boating safety course to earn a license

to pilot boats. Educational materials should be distributed to boaters during these courses. Many boaters in Barnegat Bay are not aware that terrapins live in the waters. Since diamondback terrapins do not avoid boats, humans must change their behavior to ensure continuation of terrapin populations and other aquatic wildlife.

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VITA

Lori A. Lester

3245 Chestnut St., PISB 308
Philadelphia, PA 19104
lorilester@gmail.com

Education

Ph.D., Environmental Science Drexel University, Philadelphia, PA	2012
M.E.M., Coastal Environmental Management Duke University, Durham, NC	2007
B.S., Biology, Psychology Minor Allegheny College, Meadville, PA	2005

Professional Experience

Teaching Assistant – Drexel University	2007-2012
Terrapins of Barnegat Bay – Research Staff Coordinator	2008-2010
Global Bycatch Assessment Project – Research Assistant	2006-2007

Selected Publications and Abstract

- Lester, L. A.**, E. A. Standora, W. F. Bien, and H. W. Avery. (2012). “Behavioral responses of diamondback terrapins (*Malaclemys terrapin*) to recreational boat sounds.” *The Effects of Noise on Aquatic Life*. A. N. Popper and A. Hawkins, editors. *Advances in Experimental Medicine and Biology*. Springer. 730: 361-362.
- Lester, L. A.** (10 March 2012). “Underwater anthropogenic sounds in an estuarine diamondback terrapin environment.” *Atlantic Estuarine Research Society*. Cape May, NJ.
- Lester, L. A.**, Standora, E. A., Bien, W. F., and H. W. Avery. (13 November 2010). “Hearing in Diamondback Terrapins (*Malaclemys terrapin*): The Auditory Brainstem Response Technique and Behavioral Responses to Boat Engine Sounds.” *The Fifth National Symposium on the Ecology, Status, and Conservation of the Diamondback Terrapin*. Chauvin, LA.

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- \$4,000:** Dissertation Fellowship from Drexel University’s Graduate Studies Office. Awarded on 12 June, 2012.
- \$655:** Diamondback Terrapin Working Group Research Award to Complete Underwater Hearing Tests on Diamondback Terrapins. Awarded on 8 June 2010.
- Teaching Excellence Award Nominee.** Drexel University. Awarded on 8 April 2011 and 8 May 2009.
- Poster Award.** Drexel University’s College of Arts and Sciences Research Day. Awarded 3rd place poster on 4 April 2010 and 2nd place poster on 6 April 2009.

