Received: 4 January 2023 | Revised: 27 April 2023 | Accepted: 21 May 2023
DOI: 10.1002/ar.25272

RESEARCH ARTICLE

The Anatomical Record WILEY

The anatomical basis of amphibious hearing in the American alligator (*Alligator mississippiensis*)

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Abstract

The different velocities of sound (pressure waves) in air and water make auditory source localization a challenge for amphibious animals. The American alligator (*Alligator mississippiensis*) has an extracolumellar cartilage that abuts the deep surface of the tympanic membrane, and then expands in size beyond the caudal margin of the tympanum. This extracolumellar expansion is the insertion site for two antagonistic skeletal muscles, the tensor tympani, and the depressor tympani. These muscles function to modulate the tension in the tympanic membrane, presumably as part of the well-developed submergence reflex of *Alligator*. All crocodilians, including *Alligator*, have internally coupled ears in which paratympanic sinuses connect the contralateral middle ear cavities. The temporal performance of internally coupled ears is determined, in part, by the tension of the tympanic membrane. Switching between a “tensed” and “relaxed” tympanic membrane may allow *Alligator* to compensate for the increased velocity of sound underwater and, in this way, use a single auditory map for sound localization in two very different physical environments.

KEYWORDS
audition, extracolumella, internally coupled ears, tympanic membrane

1 | INTRODUCTION

The specialized cephalic receptor for pressure waves, the tympanic membrane or eardrum, has evolved multiple times in vertebrates (e.g., Capshaw et al., 2022). Many of the evolutionary radiations of vertebrates with tympanic hearing include amphibious taxa (e.g., Clack, 2002). The auditory system of an amphibious tympanic vertebrate faces two prominent challenges: impedance matching and auditory localization. Due to the marked difference in density, the acoustic impedance of water is roughly 3500 times greater than the acoustic impedance of air (e.g., Zeqiri et al., 2010). Since all vertebrate inner ears are fluid-filled, the middle ear is often described as an “impedance matching” system (e.g., Manley & Sienknecht, 2013) in which differential areas combine with lever systems to transmit low impedance airborne pressure waves to the higher impedance cochlea. Clearly, the same differential areas and lever systems cannot impedance match pressure waves propagating in both air and water. The majority of tympanic vertebrates, and all known reptiles, localize sounds using bilateral temporal cues (e.g., Oertel, 1999; Winer & Schreiner, 2005). These bilateral temporal signals are converted into spatial cues using a variety of neural computational systems; the spatial cues, in turn, form an acoustic map of the animal's environment (e.g., Christensen-Dalsgaard & Carr, 2018; Kettler & Carr, 2019). Although this auditory localization system has been extensively studied in a variety of vertebrates (Walton et al., 2017), one aspect remains poorly understood. The pressure waves amphibious vertebrates hear in water travel 4.3 times as fast as the pressure waves they hear in air; how does the auditory system resolve this difference onto one acoustic map?

While the amount of time the American alligator (*Alligator mississippiensis*) spends on land decreases with body size (Subalusky et al., 2009), all ages can be accurately described as amphibious (e.g., Nifong & Silliman, 2013). *Alligator* is an opportunistic feeding generalist, capable of capturing prey on land, at the water surface, and underwater (e.g., Nifong et al., 2014; Shoop & Ruckdeschel, 1990; Wolfe et al., 1987). *A. mississippiensis* is capable of remaining underwater for extended periods (e.g., Uriona et al., 2019). Bierman and Carr (2015) reviewed the behavioral, physiological, and neural evidence for the detection, and localization, of airborne pressure waves by *Alligator*. Physiological and neural studies have demonstrated that the ear of *A. mississippiensis* is less sensitive underwater (Higgs et al., 2002), presumably reflecting the impedance matching issue. *Alligator*, like most crocodilians, uses acoustic communication, both in air and underwater (e.g., Vergne et al., 2009; Young et al., 2013). The use of underwater auditory signals does not indicate that the receiver can accurately localize the source; there is little behavioral evidence for underwater auditory localization in crocodilians (Dinets, 2013).

In all extant crocodilians, the skull is dorsal-ventrally flattened (Busbey & Thomason, 1995), which results in a well-defined meatal chamber on the dorso-lateral surface of the skull (Montefeltro et al., 2016). The lateral surface of the meatal chamber is covered by mobile earflaps (Shute & Bellairs, 1955; Young et al., 2022). Earlier studies have examined the earflaps; testing for possible acoustic filtering (Wever & Vernon, 1957) or alteration of the impedance matching by trapping air bubbles (Higgs et al., 2002), but no significant auditory role has been found. The middle ear cavities of crocodilians are linked by dorsal and paired ventral paratympanic passages (e.g., Dufeau & Witmer, 2015; Tahara & Larsson, 2022). The ventral paratympanic passages open into the pharynx through a muscular valve (Owen, 1850); examination suggests that this valve does not play an auditory function (Young & Bierman, 2019). Tympanic ears joined by paratympanic passages form internally coupled ears in which displacement of one tympanic membrane creates a pressure wave that propagates through the paratympanic passage to influence the contralateral tympanum (Vedurmudi, Young, & van Hemmen, 2016, Vedurmudi, Goulet, et al., 2016). Experimental analysis has confirmed the pressure coupling of the ears in *Alligator* (Bierman et al., 2014).

The present study was undertaken to describe a muscular control feature of the ear of *A. mississippiensis*. This muscular control system can modulate the internal coupling of the tympanic membranes and, by doing so, effectively "tune" the ear for airborne or aquatic pressure reception.

2 | MATERIALS AND METHODS

Nine specimens of the American alligator, *A. mississippiensis*, were examined for this study. Two of the specimens were hatchlings (total body lengths of 23–26 cm), three were juveniles (total body lengths of 63–88 cm), two were sub-adults (total body lengths of 158–178 cm), and two were adults (total body lengths of 256–296 cm). The specimens were either purchased commercially or obtained through the courtesy of the Louisiana Department of Wildlife and Fisheries. These specimens were all used for other studies, then euthanized, and the head of each specimen removed intact. The heads of the hatchling and juvenile specimens were fixed in 10% neutral-buffered formalin (nbf) for at least 48 h at 4°C; the heads of the sub-adult specimens were frozen whole, while the heads of the adult specimens were first bisected sagittally then frozen.

Frozen material was thawed overnight before dissection. The results of the dissection were documented using a digital camera (Nikon D3100) or a dissecting microscope (Leica M80) using a IC80HD digital camera (Leica).

The heads of the nbf-preserved specimens were transferred to 70% ethanol for storage. For histological analysis, the otic region of the head was removed using a bone saw, then placed in RDO Rapid Decalcifier (Apex Engineering, Aurora, IL) for 24–48 h. Once fully decalcified, the block was dehydrated through an ethanol series before paraffin embedding. Complete serial transverse, sagittal, and frontal sections were cut (at 10 μm) through the tympanic membrane and surrounding tissue. Sections were stained with hematoxylin and eosin, Masson's trichrome stain, and phosphotungstic acid hematoxylin (PTAH). Microscopic anatomy was documented using a DM 4000B microscope (Leica Microsystems Inc., Buffalo Grove, IL). The frontal sections were scanned (Aperio CS2, Leica Microsystems Inc.) then imported into BioVis3D (BioVis3D, Montevideo, Uruguay) which was used for a 3D reconstruction.

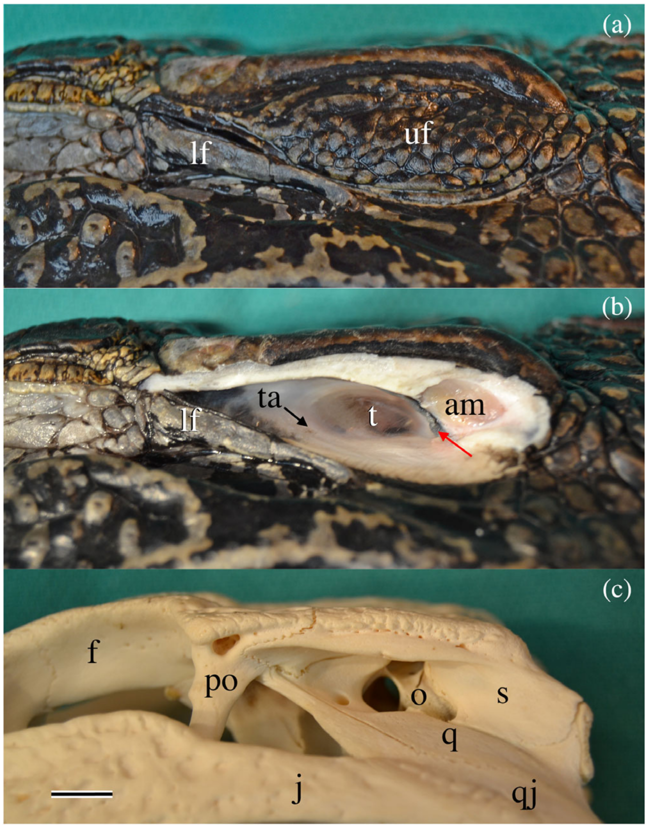

FIGURE 1 Features of the meatal chamber of *Alligator mississippiensis*. (a) The superficial covering of the meatal chamber consists of the larger, more caudal, and more mobile, upper ear flap (uf), and the smaller, more rostral, lower ear flap (lf). (b) Removal of the upper ear flap exposes some of the auricular muscles (am) which move the upper ear flap, as well as the meatal chamber. The tympanic membrane (t) is positioned nearly 40° to the horizontal and is surrounded by a tympanic annulus (ta). A portion of the highly pigmented meatal epithelium (red arrow) was removed with the upper ear flap. (c) Skull of a similar-sized *A. mississippiensis*, showing the osteological elements associated with the meatal chamber and tympanic membrane. f, frontal; j, jugal; o, otic buttress; po, postorbital; q, quadrate; qj, quadratojugal; s, squamosal. Scale bar = 1 cm.

The complete tympanic membrane, with all surrounding soft-tissue and with the stapes still attached, was removed from one juvenile specimen. The excised tissue was partially cleared then stained with Alizarin red S (Sigma-Aldrich Inc., St. Louis, MO), and Alcian blue (Sigma-Aldrich); the protocol of Wassersug (1976) was modified to shorten the clearing to keep the tympanic annulus and membrane intact and slightly opaque.

The head of one of the juvenile specimens was pre-treated in Lugol's solution then scanned at the University of Texas High-Resolution x-ray CT Facility using a custom-built North Star Imaging scanner in volume mode. The x-ray source was set to 130 kV and 0.14 mA with an aluminum foil pre-filter. A total of 3600 projections were acquired over 360 degrees of rotation, at 1 frame per second with no frame averaging and no detector binning. A beam-hardening correction of 0.25 was applied; the resulting slices measured 1979 × 1979 pixels and had a voxel resolution of 9.65 μm. The resulting DICOM images were examined using Dragonfly (Object Research Systems).

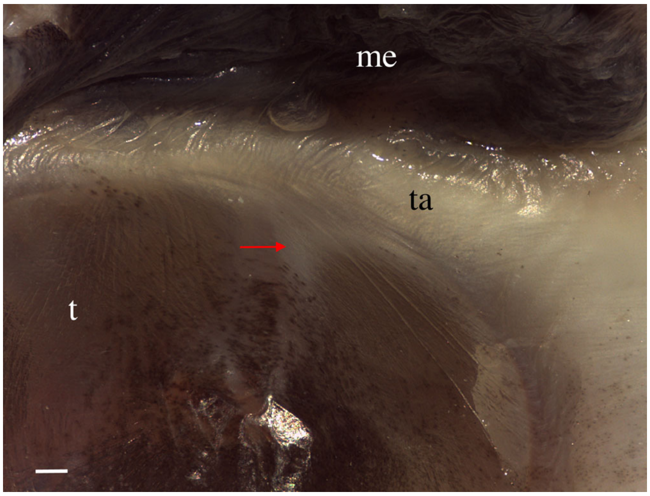

FIGURE 2 Caudal margin of the meatal chamber and tympanic membrane of a sub-adult specimen. The darkly pigmented meatal epithelium (me) is separated from the tympanic membrane (t) by a thick ring of collagenous connective tissue, the tympanic annulus (ta). A portion of the extracolumellar cartilage can be seen through the tympanic membrane (red arrow). This portion of the extracolumella extends from the apex of the tympanum caudally to course deep to the tympanic annulus. Scale bar is 500 μm.

3 | RESULTS

3.1 | Tympanic membrane

The meatal chamber of *A. mississippiensis* is shaped like a semi-ellipsoid. The lateral surface of the meatal chamber, the flat surface of the semi-ellipsoid, is covered by the mobile upper and lower ear flaps (Figure 1a). Excising the ear flaps will reveal the skeletal muscle responsible for moving the larger upper ear flap (located on the caudal margin of the meatal chamber), and the tympanic membrane (Figure 1b). The tympanum of *Alligator*, and the other extant crocodilians, is unusual in being positioned deep from the surface of the skull, and being oriented roughly 40° from the horizontal plane (Figure 1b). The tympanic membrane bulges slightly outward; the high-point of the membrane (the umbo) is located slightly caudal to the midpoint. The tympanic membrane

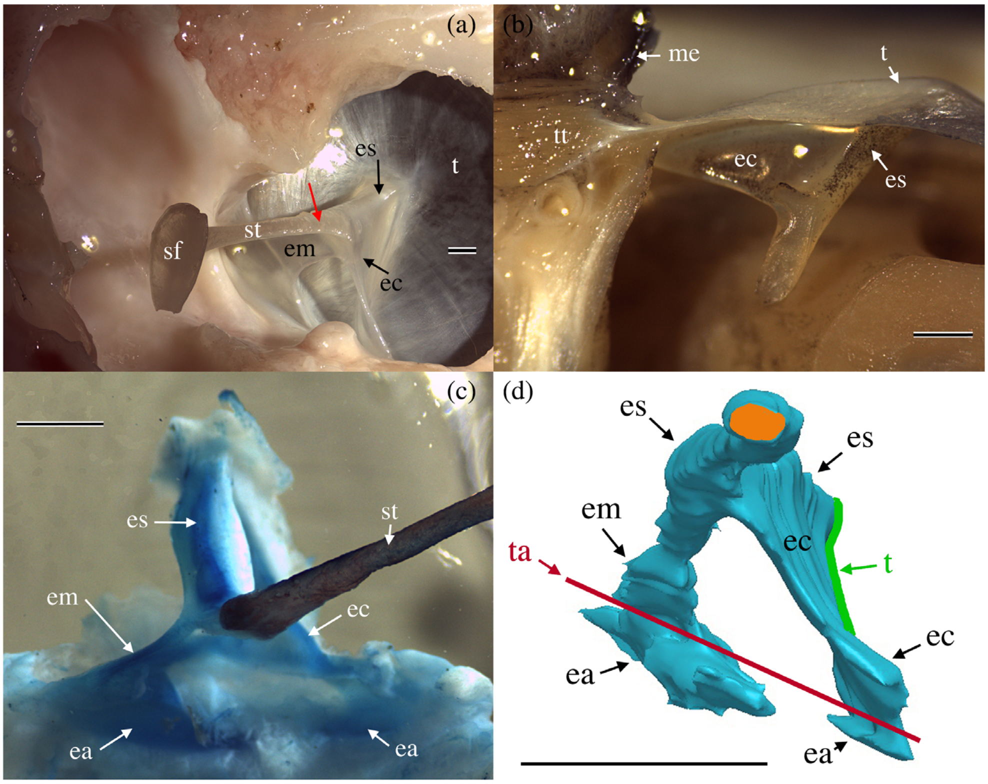

FIGURE 3 Morphology of the extracolumella of *Alligator mississippiensis*. (a) View of deep (inner) surface of the tympanic membrane (t) from the middle ear cavity of a sub-adult; the proximal end of the stapes (st) expands as the stapedial footplate (sf), while the distal end is fused to the extracolumella (red arrow). The extracolumella extends as a shaft (es) from the stapes to end as a slightly expanded head on the inner surface of the tympanic membrane. Along the way, the extracolumellar shaft supports prominent medial (em) and caudal (ec) segments, the latter of which has an extensive contact with the tympanic membrane. (b) Lateral view of a parasagittal section through the tympanic membrane (t) of a sub-adult showing the caudal (ec) portion of the extracolumella in relationship to the extracolumellar shaft (es); the caudal portion of the extracolumella extends beyond the tympanic membrane to a large skeletal muscle (the tensor tympani, tt). The caudal surface of the meatal chamber is marked by a darkly pigmented epithelium (me). (c) Partially cleared and stained preparation from a yearling specimen; the stapes (st) is bone and stains differently than the extracolumella. The extracolumellar shaft (es) extends distally to abut the deep (inferior) surface of the tympanic membrane. The medial (em) and caudal (ec) portions of the extracolumella extend away from the shaft to contact (ec) or approach (em) the tympanic annulus. Both the medial and caudal segments give rise to distal expansions that extend deep and caudal to the tympanic annulus. These large expansions, which typically fuse, form the annular segment (ea) of the extracolumella and are the functional pivot for this functional complex. (d) The 3D reconstruction of the extracolumella; the contact surface with the tympanic membrane is indicated by the green line, while the position of the tympanic annulus is shown by the red line. Note that both the medial and caudal segments of the extracolumella extend beyond the margin of the tympanum/tympanic annulus, where they expand to form the annular portion of the extracolumella. Scale bars = 1 mm.

is supported by the tympanic annulus (ta), a ring of collagenous tissue present around the complete perimeter of the membrane (Figure 1b).

As the quadratojugal courses along the caudo-medial surface of the jugal, it forms a flattened shelf of bone, the otic fossa (Figure 1c). The otic fossa is bounded caudally by a nearly vertical portion of the squamosal, which forms a low ridge that has been termed the otic buttress (Figure 1c). The tympanic annulus, and thus the tympanic membrane, is loosely attached to the margins of the otic fossa.

The meatal chamber is lined by the darkly pigmented meatal epithelium (Figure 2). The epithelium is slack and easily displaced relative to the deeper soft tissue. If the surface of the caudal portion of the tympanum is examined (Figure 2), a deeper element, the caudal portion of the extracolumella, can be seen coursing from the umbo to the tympanic annulus. At the caudal margin of the tympanic membrane, the tympanic annulus is more pronounced than it is anywhere else along the membrane's perimeter; this thickened portion of the tympanic annulus is one component of what has been termed the supporting plate of the tympanum.

3.2 | Extracolumella cartilage

In *A. mississippiensis* the extracolumella cartilage has a prominent central shaft. The proximal end of the

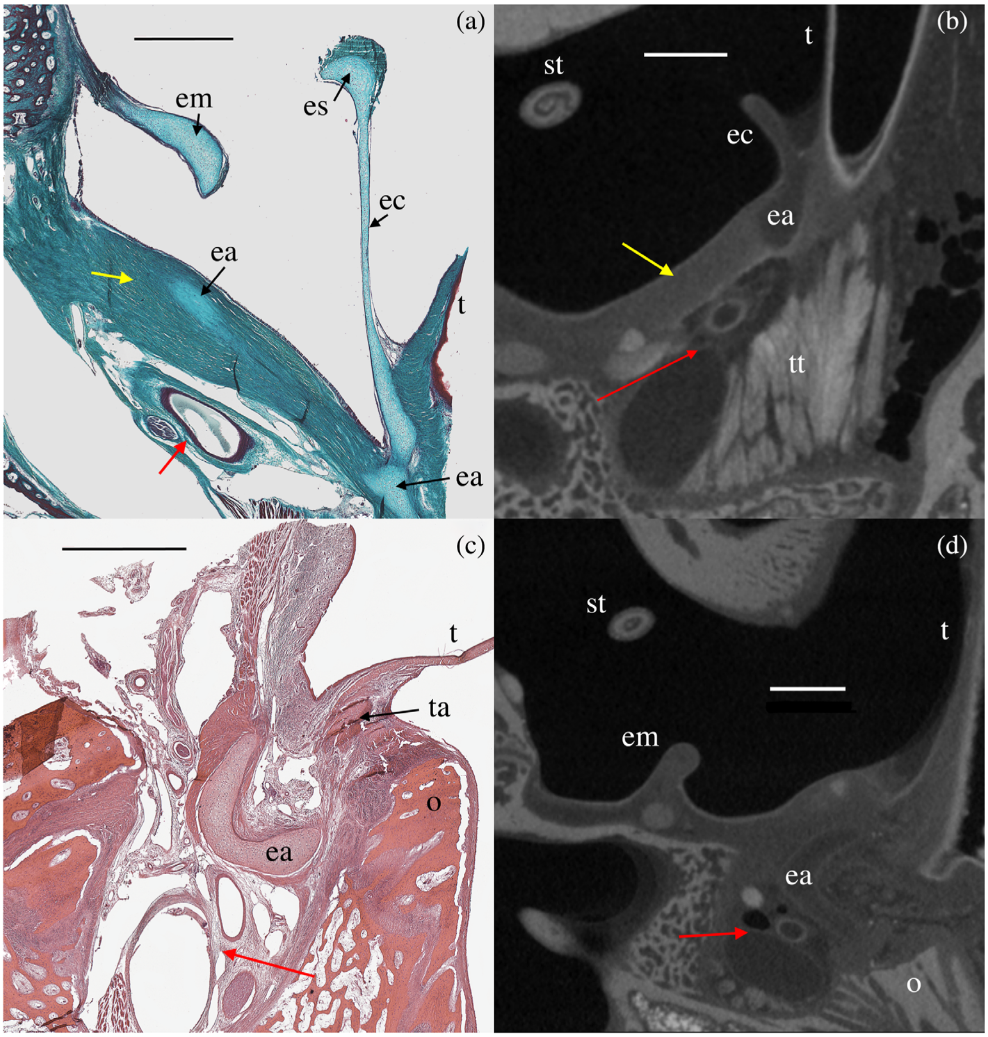

FIGURE 4 Peripheral attachments of the extracolumella in hatchling *Alligator mississippiensis*. (a) Frontal section through the extracolumella; the extracolumellar shaft (es) and caudal segment (ec) extend distally to attach to the inner (inferior) surface of the tympanic membrane (t). The medial segment of the extracolumella (em) only has indirect (collagenous) contact with the surrounding bone. The large annular segment of the extracolumella (ea) is located just rostral to a large neurovascular bundle (red arrow). The annular segment is bound to a thick band of collagenous connective tissue (yellow arrow), but has no direct attachment to the surrounding bone. (b) The micro-CT image showing a frontal section through the annular portion of the extracolumella (ea). The ea is continuous with the caudal portion (ec), bound to the collagenous band along the caudal margin of the tympanum (yellow arrow), contacted by the tensor tympani (tt), and abutted by a neurovascular bundle (red arrow). (c) Sagittal section through the annular segment of the extracolumella (ea); note the large neurovascular bundle (red arrow) located just caudal to the cartilage. The annular segment is caudal to the tympanic membrane (t) and tympanic annulus (ta), and only has indirect (collagenous) attachment to the surrounding surfaces of the otic buttress (o). (d) The micro-CT image showing a sagital section through the annular portion of the extracolumella (ea). The collagenous tissue surrounding the margin of the tympanic membrane (t) also attaches to the medial segment of the extracolumella (em), and the otic buttress (o); the ea is isolate from direct bony attachments in part by the large neurovascular bundle (red arrow). st, stapes. Scale bars = 1 mm.

extracolumellar shaft (es) articulates with the distal end of the columella or stapes (Figure 3a). The distal end of the extracolumellar shaft expands slightly and forms the strongest contact point with the tympanic membrane; this contact point is an umbo, since the extracolumellar shaft causes the tympanic membrane to bulge outward slightly. Two cartilage processes extend from the extracolumellar shaft. The medial process of the extracolumella (em) is the smaller of the two processes, it has no direct contact with the tympanic membrane, but extends medially to abut the inner surface of the tympanic annulus (Figure 3a,c). The caudal process of the extracolumella (ec) has a longer course to reach the tympanic annulus. While the medial process is more of a rod, the caudal process is a plate or sheet with a deep-to-superficial extent that creates a nearly continuous contact surface with the tympanic membrane from the umbo to the tympanic annulus on the caudal margin of the tympanum (Figure 3a,b).

As the medial and caudal processes of the extracolumella reach the level of the tympanic annulus, they expand. The medial process expands mainly in the lateral direction, while the caudal process expands mainly in the dorsal direction (with a characteristic "C" shape in sagittal section, Figure 4). The expanded distal ends of the medial and caudal extracolumellar processes frequently fuse (Figure 3c,d), forming a structure termed the annular process (ea). The extracolumellar cartilage of *A. mississippiensis* can be envisioned as a triangle; with the base (the annular process) deep to the tympanic annulus, and the apex formed by the thickened extracolumellar shaft (Figure 3c,d).

The annular process of the extracolumella is embedded in a thick band of dense regular connective tissue that occurs along the caudal margin of the tympanic membrane (Figure 4). The annular process is not directly attached to the otic buttress; but there is an indirect (collagenous) connection between the two. There is a prominent neurovascular bundle that courses along the caudal surface of the annular process (Figure 4).

3.3 | Tympanic musculature

The tympanic complex in *A. mississippiensis* includes two skeletal muscles. The tensor tympani (tt) originates from the caudal margin of the otic fossa, course craniad, and inserts on both the caudal margin of the tympanic membrane and the annular process of the extracolumella (Figure 5a,b). The insertion site on the annular process of the extracolumella is near the expanded transition between the caudal and annular processes (Figures 3 and 5). The tensor tympani is innervated by the facial nerve (CN VII); this identification is based on our dissections and the atlas of *Alligator* cranial nerves (Lessner & Holliday, 2022).

The second skeletal muscle of the tympanic complex is the depressor tympani (dt). This muscle appears to be a specialized slip of the larger depressor auriculae superior, it originates from the squamosal, courses ventrally, then has a tendinous insertion onto the dorsal tip of the annular process of the extracolumella (Figure 5c,d). This muscle, and the tendon of insertion, is located just deep to the pigmented meatal epithelium (me) along the caudal margin of the meatal chamber (Figure 5c,d). The depressor tympani is innervated by the facial nerve (CN VII); this identification is based on our dissections and the atlas of *Alligator* cranial nerves (Lessner & Holliday, 2022).

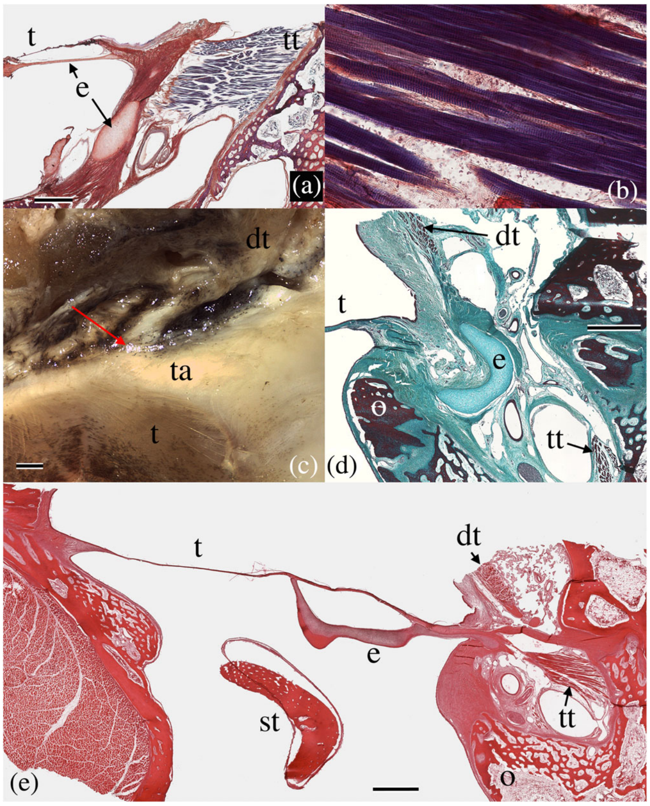

FIGURE 5 Tympanic musculature of *Alligator mississippiensis*. (a) Sagittal section through the caudal end of the tympanic membrane of a hatchling, with the tensor tympani (tt) inserting on both the extracolumella (e) and the tympanic membrane (t). (b) Phosphotungstic acid Hematoxylin (PTAH) stain of the tensor tympani muscle from a yearling specimen showing the clear striations of the skeletal muscle fibers. (c) Dissection of the caudal wall of the meatal chamber of an adult specimen, the large tendon of the depressor tympani (dt) inserts onto the extracolumella (red arrow) just caudal to the tympanic annulus (ta). (d) Sagittal section through the caudal end of a hatchling's tympanic membrane where the expansion of the extracolumella (e) rests on the otic buttress (o) and serves as an insertion site for both the depressor tympani (dt) and the tensor tympani (tt). (e) Sagittal section through the entire tympanic membrane (t) of a hatchling specimen. The tympanic membrane is fused to the extracolumella (e); at the caudal margin of the meatal chamber the depressor tympani (dt) and tensor tympani (tt) approach in opposite directions to insert on the extracolumella. The bony stapes (st) is shown near its point of fusion to the cartilaginous extracolumella. Scale bar = 1 mm.

The depressor tympani is smaller than the tensor tympani (Figure 5e). Though these two muscles are near one another, their insertions are on opposite surfaces of the expanded annular process (Figure 5e). In this way, the tensor tympani and depressor tympani form an antagonistic functional pair to modulate tympanic tension.

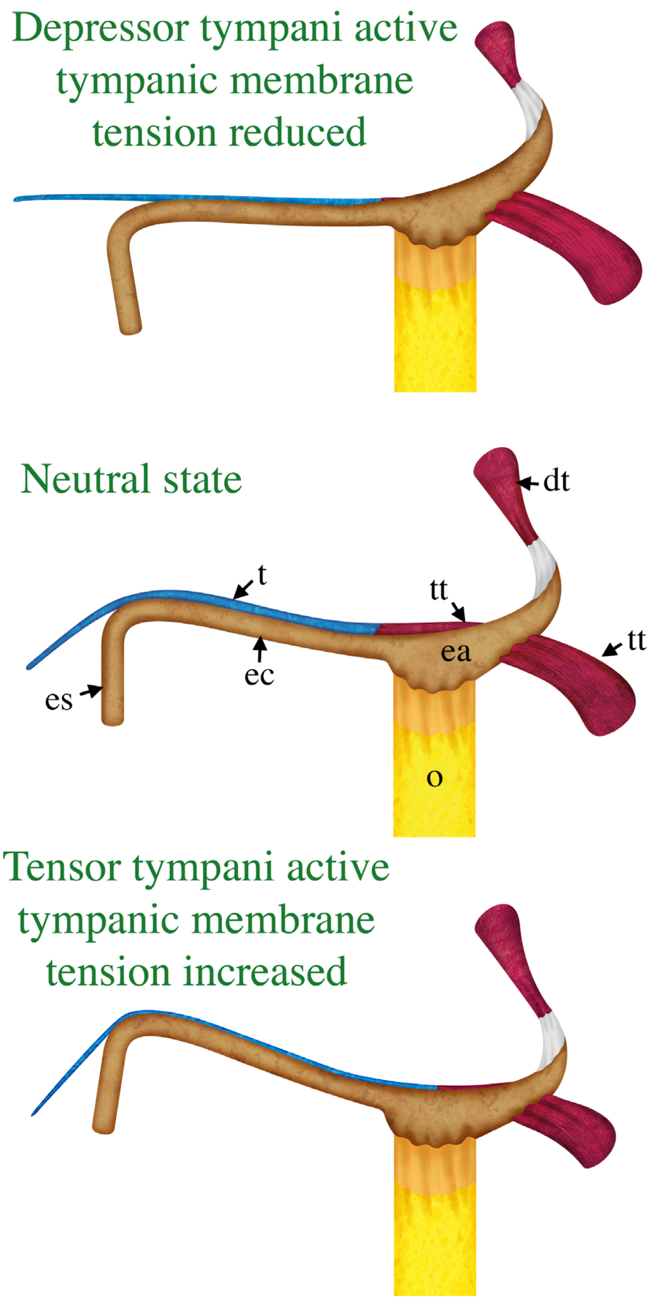

FIGURE 6 Schematic drawing of the anatomical basis for regulation of tympanic tension in *Alligator mississippiensis*. The extracolumellar shaft (es) supports the large caudal segment of the extracolumella (ec) which is attached to the inner surface of the tympanic membrane (T). At the caudal margin of the tympanum, where the tympanic annulus is surrounded by other collagenous tissue (orange), the caudal segment expands as the annular segment of the extracolumella (ea). The annular segment is the site of insertion of two skeletal muscles with opposite lines of action, the depressor tympani (dt) and the tensor tympani (tt). The annular segment of the extracolumella is not directly bound to the deep bony surface of the otic buttress (o), but instead can pivot at this point, thereby displacing the caudal segment of the extracolumella and the attached tympanic membrane. Note that the displacement of the extracolumella (ea) and the associated changes in the tympanic membrane (t), are exaggerated for clarity.

3.4 | Functional model

Herein it is proposed that the tympanic complex of *A. mississippiensis* functions to regulate the tension of the tympanic membrane. Contraction of the tensor tympani (tt) exerts a ventro-caudal force on the caudal tip of the annular process of the extracolumella. When this force is applied the extracolumella functions as a lever; the caudal end is displaced ventrally, while the umbo displaces dorsally (thereby increasing the tension of the tympanic membrane, Figure 6). In this lever system the pivot point is near the tympanic annulus where the extracolumella is embedded in a band of collagenous connective tissue. This tensing function is enhanced by the direct connection between the tensor tympani and the caudal margin of the tympanic membrane (Figures 5 and 6).

Contraction of the depressor tympani would exert a force on the dorsal tip of the annular process of the extracolumella. When this force is applied the extracolumella functions as a lever; the caudal end is displaced dorsally, while the umbo displaces ventrally (thereby decreasing the tension of the tympanic membrane, Figure 6). In this functional model the medial process of the extracolumella is seen primarily as a secondary anchor that functions to minimize rotational displacement of the extracolumella in the frontal plane.

4 | DISCUSSION

The muscle herein identified as the tensor tympani (tt) has been previously noted by multiple authors (e.g., Huxley, 1869; Killian, 1890; Peters, 1868; Wever, 1978), and often termed the stapedius. Killian (1890) described a particularly complex stapedius muscle in *Crocodylus acutus*, in part by treating the portion of the muscle that contacted the tympanic membrane separately. Wever (1978) explicitly rejected Killian's description of the muscle, arguing that the actual morphology was far “simpler.”

The muscle herein identified as the depressor tympani (dt) does not appear to have been previously described. As noted above, this muscle appears to be a specialized slip of the depressor auriculae superior, which has been previously described (e.g., Killian, 1890; Shute & Bellairs, 1955; Wever, 1978). Furthermore, previous workers have noted slips of skeletal muscle (in this same region) that were interpreted as functioning to tense or adjust the epithelial lining of the meatal chamber (e.g., Shute & Bellairs, 1955); it seems quite possible that the depressor tympani was treated as one of the meatal epithelial tensors by previous authors.

As numerous previous authors have noted (e.g., Frank & Smit, 1974; Kundrat et al., 2009), the columella (stapes)/extracolumella in crocodilians has long suffered from confusion, a plethora of synonyms, and assumed homology. Indeed, Huxley (1869) devoted much of his contribution to disputing a previous claim that one of the extracolumellar processes was homologous to the malleus. This contribution used anatomical position to name the processes of the extracolumella, in part for clarity, but also to avoid assumptions of homology which are beyond the scope of this article. Nomenclature aside, it is easy to match our description of the extracolumella of *Alligator* with the description provided by Wever (1978). The only aspect of the extracolumellar morphology that appears to be unique to this contribution is the finding that in some specimens the annular process of the extracolumella, which Wever (1978) described as the suprastapedial cartilage, forms a continuous element linking the distal tips of the medial and caudal processes of the extracolumella. This may reflect interspecific or even ontogenetic variation.

A key part of the functional model proposed herein is the extracolumella forming a lever with the otic buttress as the fulcrum (Figure 6). For this system to function as depicted, the annular process of the extracolumella has to be supported yet still relatively free of the deeper otic buttress. Killian (1890) described the band of collagenous tissue present at this spot (Figure 4); Montefeltro et al. (2016) followed Wever (1978) in referring to this collagenous band as the "supporting plate."

Herein it is proposed that the combination of the extracolumellar cartilage and tympanic muscles functions as a mechanism to adjust/modulate the tympanic tension. This is not a novel hypothesis. Killian (1890) performed the same simple procedure we did on the fresh material we examined, and noted, "If you grasp the [tensor tympani] with tweezers and pull on it, it moves the limbus of the tympanic membrane, and tenses the membrane in the direction of the pull" (translation BAY). Wever (1978) questioned Killian's interpretation, though this seemed more based on the Killian's presentation of the muscular morphology, rather than the functional hypothesis. Our interpretation aligns with Killian's; by adding the depressor tympani muscle (which Killian did not describe), the ability of this system to modulate tympanic tension is even clearer.

Alligator has a well-developed submergence reflex with bradycardia and apnea (Andersen, 1961; Axelsson & Franklin, 2011; Campbell et al., 2010; Wilber, 1960). There is also a suite of cephalic submergence reflexes including the external nares (Klassen et al., 2020) the eyelids (Garrick & Saiff, 1974; Young et al., 2022) and the ear flaps (Shute & Bellairs, 1955). We hypothesize that in addition to the earflaps, eyelids, and external nares (all of which are modulated by antagonistic skeletal muscles, and some of which are also innervated by the facial nerve), there is a submergence reflex at the tympanum. More specifically, we hypothesize that submergence results in activation of the tensor tympani muscle and an increase in tympanic tension.

A reflexive change in tympanic tension upon submergence of *A. mississippiensis* would exploit one of the properties of internally coupled ears. The size and shape of the paratympanic passages through the skull are one of the main determinants of the temporal properties of internally coupled ears (Vedurmudi, Young, & van Hemmen, 2016, Vedurmudi, Goulet, et al., 2016). These paratympanic passages are fixed, but there are a variety of mechanisms that an animal can use to modulate or tune the performance of internally-coupled ears (Young, 2016), including altering tympanic tension.

The relationship between tympanic tension and internally coupled ears was explored in relation to another amphibious reptile, the water monitor lizard (*Varanus salvator*). The water monitor has a tympanus muscle, the contraction of which increases the tension of the tympanic membrane (Han & Young, 2016). The influence of this tympanic tension on auditory performance was subsequently modeled (Vedurmudi et al., 2020); one of the main metrics studied was the time dilation factor (TDF) which is simply the temporal difference of the tympana in internally coupled ears divided by the temporal difference of the tympana in independent ears. The analysis concluded that, "there is a decrease in the TDF with an increase in membrane tension" (Vedurmudi et al., 2020). For *Varanus* the change in TDF was predicted to be around 2 (Vedurmudi et al., 2020). The tympanus muscle of *Varanus* is much smaller than the tensor tympani of *Alligator* (though the specimens of *V. salvator* studied were over 150 cm long) suggesting that in *Alligator* greater tympanic tensions could be generated, with correspondingly greater decreases in TDF. If the TDF value varied by just over 4 upon contraction of the tensor tympani, then the tympanic complex of *Alligator* could be quickly (and reflexively) adjusted or tuned to the 4.3 × greater velocity of pressure waves in water. In this way the alligator could accurately use the same auditory map for sound localization both in air and in water.

It is not our intention to argue that the TDF of the auditory system must perfectly offset the differential velocity of sound in air and water; that is equivalent to arguing that the precision of aquatic sound localization by *A. mississippiensis* must be exactly the same as the precision of airborne sound localization. Crocodilians have specialized multimodal trigeminal sense organs termed domed pressure receptors (Di-Poï & Milinkovitch, 2013; Grap et al., 2020). Switching from audition (far-field) to domed pressure receptors (near-field) would allow the crocodilian to easily compensate for any error/ambiguity in sound source localization. Sensory modality switches of this type are well known among vertebrates, especially aquatic taxa (Braithwaite & De Perera, 2006; Collin & Davies, 2020; Gardiner et al., 2014).

The differences in the physical environments occupied by an amphibious vertebrate place significant functional demands on the auditory system (see Gridi-Papp & Narins, 2009; Hetherington, 2008). Some amphibious vertebrates have specialized for hearing in one medium (e.g., underwater) at the expense of auditory performance in the other medium (Christensen-Dalsgaard et al., 2012). The results of this study suggest that by evolving the capacity to modulate tympanic membrane tension, *Alligator* may have a tympanic ear capable of effectively functioning both in air and underwater.

AUTHOR CONTRIBUTIONS

Bruce A. Young: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; formal analysis; project administration; data curation; supervision; resources. **Michael Cramberg:** Investigation; methodology; visualization; writing – review and editing; formal analysis.

ACKNOWLEDGEMENTS

The authors wish to thank the Louisiana Department of Wildlife and Fisheries for their cooperation, and P. Kondrashov for his continued support. Figure 6 was skillfully rendered by Jamie Carroll.

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REFERENCES

Andersen, H. T. (1961). Physiological adjustments to prolonged diving in the American alligator *Alligator mississippiensis*. *Acta Physiologica Scandinavica*, *53*, 23–45.

Axelsson, M., & Franklin, C. E. (2011). Elucidating the responses and role of the cardiovascular system in crocodilians during diving: Fifty years on from the work of CG Wilber. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *160*, 1–8.

Bierman, H. S., & Carr, C. E. (2015). Sound localization in the alligator. *Hearing Research*, *329*, 11–20.

Bierman, H. S., Thornton, J. L., Jones, H. G., Koka, K., Young, B. A., Brandt, C., Christensen-Dalsgaard, J., Carr, C. E., & Tollin, D. J. (2014). Biophysics of directional hearing in the American alligator (*Alligator mississippiensis*). *Journal of Experimental Biology*, *217*, 1094–1107.

Braithwaite, V. A., & De Perera, T. B. (2006). Short-range orientation in fish: How fish map space. *Marine and Freshwater Behaviour and Physiology*, *39*, 37–47.

Busbey, A. B., & Thomason, J. J. (1995). The structural consequences of skull flattening in crocodilians. In J. J. Thomason (Ed.), *Functional morphology in vertebrate paleontology* (pp. 173–192). Cambridge University Press.

Campbell, H. A., Sullivan, S., Read, M. A., Gordos, M. A., & Franklin, C. E. (2010). Ecological and physiological determinants of dive duration in the freshwater crocodile. *Functional Ecology*, *24*, 103–111.

Capshaw, G., Christensen-Dalsgaard, J., & Carr, C. E. (2022). Hearing without a tympanic ear. *Journal of Experimental Biology*, *225*, jeb244130.

Christensen-Dalsgaard, J., Brandt, C., Willis, K. L., Christensen, C. B., Ketten, D., Edds-Walton, P., Fay, R. R., Madsen, P. T., & Carr, C. E. (2012). Specialization for underwater hearing by the tympanic middle ear of the turtle, *Trachemys scripta elegans*. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 2816–2824.

Christensen-Dalsgaard, J., & Carr, C. (2018). Processing of directional information in the gecko auditory nerve. *Acta Acustica United with Acustica*, *104*, 848–851.

Clack, J. A. (2002). Patterns and processes in the early evolution of the tetrapod ear. *Journal of Neurobiology*, *53*, 251–264.

Collin, S. P., & Davies, W. I. (2020). Biodiversity of sensory systems in aquatic vertebrates. *Frontiers in Ecology and Evolution*, *8*, 192.

Dinets, V. (2013). Underwater sound locating capability in the American alligator (*Alligator mississippiensis*). *Journal of Herpetology*, *47*, 521–523.

Di-Poï, N., & Milinkovitch, M. C. (2013). Crocodylians evolved scattered multi-sensory micro-organs. *EvoDevo*, *4*, 1–16.

Dufeau, D. L., & Witmer, L. M. (2015). Ontogeny of the middle-ear air-sinus system in *Alligator mississippiensis* (Archosauria: Crocodylia). *PLoS One*, *10*, e0137060.

Frank, G. H., & Smit, A. L. (1974). The early ontogeny of the columella auris of *Crocodilus niloticus* and its bearing on problems concerning the upper end of the reptilian hyoid arch. *African Zoology*, *9*, 59–87.

Gardiner, J. M., Atema, J., Hueter, R. E., & Motta, P. J. (2014). Multisensory integration and behavioral plasticity in sharks from different ecological niches. *PLoS One*, *9*, e93036.

Garrick, L. D., & Saiff, E. I. (1974). Observations on submergence reflexes of *Caiman sclerops*. *Journal of Herpetology*, *8*, 231–235.

Grap, N. J., Machts, T., Essert, S., & Bleckmann, H. (2020). Stimulus discrimination and surface wave source localization in crocodilians. *Zoology*, *139*, 125743.

Gridi-Papp, M., & Narins, P. M. (2009). Environmental influences in the evolution of tetrapod hearing sensitivity and middle ear tuning. *Integrative and Comparative Biology*, *49*, 702–716.

Han, D., & Young, B. A. (2016). Anatomical basis of dynamic modulation of tympanic tension in the water monitor lizard, *Varanus salvator*. *The Anatomical Record*, *299*, 1270–1280.

Hetherington, T. (2008). Comparative anatomy and function of hearing in aquatic amphibians, reptiles, and birds. In J. G. M. Thewissen & S. Nummela (Eds.), *Sensory evolution on the threshold: Adaptations in secondarily aquatic vertebrates* (pp. 183–209). University of California Press.

Higgs, D., Brittan-Powell, E., Soares, D., Souza, M., Carr, C., Dooling, R., & Popper, A. (2002). Amphibious auditory responses of the American alligator (*Alligator mississipiensis*). *Journal of Comparative Physiology A*, *188*, 217–223.

Huxley, T. H. (1869). On the representatives of the malleus and the incus of the Mammalia in the other vertebrata. *Proceedings of the Zoological Society of London*, *27*, 391–407.

Kettler, L., & Carr, C. E. (2019). Neural maps of interaural time difference in the American alligator: A stable feature in modern archosaurs. *Journal of Neuroscience*, *39*, 3882–3896.

Killian, G. (1890). Die ohrmuskeln des krokodiles nebst vorläufigen bemerkungen über die homologie des musculus stapedius und des stapes. *Jenaische Zeitschrift für Naturwissenschaft*, *24*, 632–656.

Klassen, M., Adams, J., Cramberg, M., Knoche, L., & Young, B. A. (2020). The narial musculature of *Alligator mississippiensis*: Can a muscle be its own antagonist? *Journal of Morphology*, *281*, 608–619.

Kundrat, M., Janáček, J., & Russell, A. P. (2009). Developmental patterns of the crocodilian and avian columella auris: Reappraisal of interpretations of the derivation of the dorsal hyoid arch in archosaurian tetrapods. *Zoological Journal of the Linnean Society*, *156*, 384–410.

Lessner, E. J., & Holliday, C. M. (2022). A 3D ontogenetic atlas of Alligator mississippiensis cranial nerves and their significance for comparative neurology of reptiles. *Anatomical Record*, *305*, 2854–2882.

Manley, G. A., & Sienknecht, U. J. (2013). The evolution and development of middle ears in land vertebrates. In S. Puria, R. R. Fay, & A. N. Popper (Eds.), *The middle ear: Science, otosurgery, and technology* (pp. 7–30). Springer.

Montefeltro, F. C., Andrade, D. V., & Larsson, H. C. (2016). The evolution of the meatal chamber in crocodyliforms. *Journal of Anatomy*, *228*, 838–863.

Nifong, J. C., Nifong, R. L., Silliman, B. R., Lowers, R. H., Guillette, L. J., Jr., Ferguson, J. M., Welsh, M., Abernathy, K., & Marshall, G. (2014). Animal-borne imaging reveals novel insights into the foraging behaviors and diel activity of a large-bodied apex predator, the American alligator (*Alligator mississippiensis*). *PLoS One*, *9*, e83953.

Nifong, J. C., & Silliman, B. R. (2013). Impacts of a large-bodied, apex predator (*Alligator mississippiensis* Daudin 1801) on salt marsh food webs. *Journal of Experimental Marine Biology and Ecology*, *440*, 185–191.

Oertel, D. (1999). The role of timing in the brain stem auditory nuclei of vertebrates. *Annual Review of Physiology*, *61*, 497–519.

Owen, R. (1850). On the communications between the cavity of the tympanum and the palate in the Crocodilia (gavials, alligators and crocodiles). *Philosophical Transactions of the Royal Society of London*, *140*, 521–527.

Peters, W. (1868). On the tympanic bone and ossicles of the ear in the Monotremata, in connexion with the question of the interpretation of the quadrate bone in birds. *Journal of Natural History*, *1*, 390–392.

Shoop, C. R., & Ruckdeschel, C. A. (1990). Alligators as predators on terrestrial mammals. *American Midland Naturalist*, *124*, 407–412.

Shute, C. C. D., & Bellairs, A. D. A. (1955). The external ear in Crocodilia. *Proceedings of the Zoological Society of London*, *124*, 741–749.

Subalusky, A. L., Fitzgerald, L. A., & Smith, L. L. (2009). Ontogenetic niche shifts in the American alligator establish functional connectivity between aquatic systems. *Biological Conservation*, *142*, 1507–1514.

Tahara, R., & Larsson, H. C. (2022). Paratympanic sinuses in juvenile alligator. *The Anatomical Record*, *305*, 2926–2979.

Uriona, T. J., Lyon, M., & Farmer, C. G. (2019). Lithophagy prolongs voluntary dives in American alligators (*Alligator mississippiensis*). *Integrative Organismal Biology*, *1*, oby008.

Vedurmudi, A. P., Goulet, J., Christensen-Dalsgaard, J., Young, B. A., Williams, R., & van Hemmen, J. L. (2016). How internally coupled ears generate temporal and amplitude cues for sound localization. *Physical Review Letters*, *116*, 028101.

Vedurmudi, A. P., Young, B. A., & van Hemmen, J. L. (2016). Internally coupled ears: Mathematical structures and mechanisms underlying ICE. *Biological Cybernetics*, *110*, 359–382.

Vedurmudi, A. P., Young, B. A., & van Hemmen, J. L. (2020). Active tympanic tuning facilitates sound localization in animals with internally coupled ears. *Hearing Research*, *387*, 107861.

Vergne, A. L., Pritz, M. B., & Mathevon, N. (2009). Acoustic communication in crocodilians: From behaviour to brain. *Biological Reviews*, *84*, 391–411.

Walton, P. L., Christensen-Dalsgaard, J., & Carr, C. E. (2017). Evolution of sound source localization circuits in the nonmammalian vertebrate brainstem. *Brain, Behavior and Evolution*, *90*, 131–153.

Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technology*, *51*, 131–134.

Wever, E. G. (1978). *The reptile ear: Its structure and function*. Princeton University Press.

Wever, E. G., & Vernon, J. A. (1957). Auditory responses in the spectacled caiman. *Journal of Cellular and Comparative Physiology*, *50*, 333–339.

Wilber, C. G. (1960). Cardiac responses of *Alligator mississippiensis* to diving. *Comparative Biochemistry and Physiology*, *1*, 164–166.

Winer, J. A., & Schreiner, C. E. (2005). The central auditory system: A functional analysis. In J. A. Winer & C. E. Schreiner (Eds.), *The inferior colliculus* (pp. 1–68). Springer.

Wolfe, J. L., Bradshaw, D. K., & Chabreck, R. H. (1987). Alligator feeding habits: New data and a review. *Gulf of Mexico Science*, *9*, 1.

Young, B. A. (2016). Anatomical influences on internally coupled ears in reptiles. *Biological Cybernetics*, *110*, 255–261.

Young, B. A., & Bierman, H. S. (2019). On the median pharyngeal valve of the American alligator (*Alligator mississippiensis*). *Journal of Morphology*, *280*, 58–67.

Young, B. A., Grondel, B., Preston, P., & Cramberg, M. (2022). The orbitalauricular chord of alligator: The unusual functional linkage between the earflap and eyelid of Crocodylians. *Journal of Anatomy*, *241*, 1399–1408.

Young, B. A., Mathevon, N., & Tang, Y. (2013). Reptile auditory neuroethology: What do reptiles do with their hearing? In C. Koppl, G. A. Manley, A. N. Popper, & R. R. Fay (Eds.), *Insights from comparative hearing research* (pp. 323–346). Springer.

Zeqiri, B., Scholl, W., & Robinson, S. P. (2010). Measurement and testing of the acoustic properties of materials: A review. *Metrologia*, *47*, S156–S171.

How to cite this article: Young, B. A., & Cramberg, M. (2024). The anatomical basis of amphibious hearing in the American alligator (*Alligator mississippiensis*). *The Anatomical Record*, *307*(1), 198–207. https://doi.org/10.1002/ar.25272